EVALUATION OF EFFECTIVENESS OF VITAMIN C FOLLOWING PHASE I THERAPY BY ESTIMATING SALIVARY SUPEROXIDE DISMUTASE LEVEL IN SMOKERS WITH CHRONIC PERIODONTITIS

A Dissertation submitted in
partial fulfillment of the requirements
for the degree of

MASTER OF DENTAL SURGERY

BRANCH – II
PERIODONTICS

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
Chennai – 600 032

2010 - 2013

CERTIFICATE

This is to certify that **Dr. K.KIRUPA**, Post Graduate student (2010-2013) in the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai - 600 003, has done this dissertation titled " EVALUATION OF EFFECTIVENESS OF VITAMIN C FOLLOWING PHASE I THERAPY BY ESTIMATING SALIVARY SUPEROXIDE DISMUTASE LEVEL IN SMOKERS WITH CHRONIC PERIODONTITIS" under our direct guidance and supervision in partial fulfillment of the regulations laid down by the **Tamil Nadu Dr.M.G.R. Medical University**, Chennai - 600 032 for **M.D.S.,** (Branch-II) **Periodontics** degree examination.

Dr.S. Kalaivani
Professor and Guide

Dr.K.Malathi
Professor & H.O.D.

Department of Periodontics
Tamil Nadu Government Dental College and Hospital, Chennai - 3.

Dr. K.S.G.A. NASSER
PRINCIPAL

Tamil Nadu Government Dental College and Hospital, Chennai - 600 003

ACKNOWLEDGEMENT

I express my hearty thanks to my Guide **Dr. S. KALAIVANI M.D.S.**, Professor, Department of Periodontics, Tamil Nadu Government Dental College and Hospital, for her encouragement, support, guidance and everlasting mother-hood care during my course of PG .

I am extremely grateful to **Dr. K. MALATHI M.D.S.,** Professor & H.O.D., Department of Periodontics, and **Dr. MAHEASWARI RAJENDRAN M.D.S.,** Professor**,** Department of Periodontics, Tamil Nadu Government Dental College and Hospital, for their valuable guidance, support and encouragement during my course.

My humble thanks to **Dr. K.S.G.A. NASSER M.D.S.,** Principal, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 for his kind permission and encouragement.

My sincere thanks to **Dr. M. JEEVAREKHA M.D.S., Dr. A. MUTHUKUMARASWAMY M.D.S., Dr. P. KAVITHA M.D.S.**, Assistant professors, Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 for their valuable suggestions, constant encouragement and timely help rendered throughout my course.

I extend my sincere thanks to **Dr. PRAGNA .B. DOLIA M.D., Director**, Department of Biochemistry, Madras Medical College and Hospital, Chennai – 600 003 for granting me permission to conduct this study and her supportive guidance and avail the lab facilities throughout this study.

I wish to express my sincere thanks to **Dr. T. RAMESWARI ,** PG student, Institute of Biochemistry, Madras Medical College and Hospital, Chennai who took time off her busy schedule and expertly helped in my biochemical analysis.

I wish to thank **Dr. S. SUDHARSHINI.**, PG student Institute of Community Medicine for helping me with the statistical work of my dissertation.

I take this opportunity to express my gratitude to my colleagues, especially to **DR.S.KRITHIKA, DR.SHRUTI BERI AND DR.A.LOGARANI** and all my well wishers for their valuable help and suggestions throughout my course.

A special thanks to all my patients for their consent, co-operation and participation in this study.

I dedicate this work to my husband **Dr. P. SENTHILNATHAN M.D.S,** my daughter, my mother-in-law, my parents and my sisters for their love, care, support and prayers to overcome all my hardships and relieving me from responsibilities and giving way to make up with my course.

All glory and honor to **THE LORD ALMIGHTY** who gives me the strength to persist against all odds, whose loving kindness and mercies endureth forever.

ABSTRACT

BACKGROUND:

Smoking is an important risk factor for chronic periodontitis (ChP), that induces oxidative stress in the body resulting in an imbalance between Reactive Oxygen Species (ROS) and antioxidants. Use of antioxidant supplementations in humans as an adjunct to periodontal therapy is being one of the innovative strategies in periodontal field..

AIM:

To assess the salivary dismutase levels in predicting the effectiveness of vitamin C supplementation in phase I therapy in smokers and non smokers with ChP.

MATERIALS AND METHODS:

In the present study, a total of 70 male subjects were included, among them 10 were periodontally healthy controls (group I), remaining 60 subjects with chronic periodontitis were divided into two groups, Group II non-smokers with chronic periodontitis and Group III smokers with chronic periodontitis consisting of 30 subjects in each group. Group II and group III were further divided based on the interventional treatment provided. Subjects treated with SRP alone were designated as group IIA and group IIIA and those treated with SRP and adjunct vitamin C were designated as group IIB and group IIIB. The salivary SOD levels were measured at baseline and after one month of treatment using spectrophotometry.

RESULTS:

Baseline salivary SOD level was significantly less in smokers than non-smokers with ChP as compared to control group. There was a significant reduction in all clinical parameters in smokers and non-smokers with ChP one month following SRP. Salivary SOD level significantly increased ($p<0.01$) in smokers and non-smokers with ChP one month following SRP supplemented with vitamin C.

CONCLUSION:

Adjunctive dose of vitamin C has improved salivary SOD level in smokers and non-smokers with chronic periodontitis than SRP alone. Further longitudinal studies may be needed for the administration of vitamin C as an adjunct to periodontal therapy in order to maintain a stable periodontium.

Key words: Superoxide Dismutase, Vitamin C, Chronic Periodontitis

DECLARATION

TITLE OF DISSERTATION	Evaluation of effectiveness of vitamin C following phase I therapy by estimating salivary superoxide dismutase level in smokers with chronic periodontitis.
PLACE OF STUDY	Tamil Nadu Government Dental College & Hospital, Chennai-600003.
DURATION OF THE COURSE	3 Years
NAME OF THE GUIDE	Dr.S Kalaivani
HEAD OF THE DEPARTMENT	Dr.K.Malathi

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance/any promotion without obtaining prior permission of the Principal, Tamil Nadu Government Dental College & Hospital, Chennai-600003. In addition, I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in dissertation. The author has the right to reserve for publish of work solely with the prior permission of the Principal, Tamil Nadu Government Dental College & Hospital, Chennai-600003.

Head of the Department **Guide** **Signature of the candidate**

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And

Dr. S. Kalaivani aged 56 years working as **Professor** in Department of Periodontics at the college, having residence address No: 13 Mother Teresa street, Srinagar colony, Thirumullaivoyal, Chennai – 62 (herein after referred to as the 'Principal Investigator')

And

Dr. K. Kirupa aged 27 years currently studying as **Post Graduate student** in Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003, (hereafter referred to as 'the PG student and co-investigator')

Whereas the PG student as part of her curriculum undertakes to research on "EVALUATION OF EFFECTIVENESS OF VITAMIN C FOLLOWING PHASE I THERAPY BY ESTIMATING SALIVARY SUPEROXIDE DISMUTASE LEVEL IN SMOKERS WITH CHRONIC PERIODONTITIS" for which purpose the Principal Investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co – investigator

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Tamil Nadu Government Dental College and Hospital, Chennai-3

Telephone No: 044 2534 0343
Fax : 044 2530 0681

R.C.No. 0430/DE/2010 Date: 07-03-2012

Title of the Work **"Evluation of effectiveness of Vitamin C following Phase I therapy by estimating salivary superoxide dismutase level in smokers with chronic periodontitis"**

Principal Investigator: Dr. K. Kirupa, P.G. II Year Student

Department Department of Periodontics

The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the **IEC meeting held on 25-01-2012** at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

"Advise to proceed with the study"

The Members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the Principal Investigator.

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S Jayachand 07/03/12

SECRETARY

CHAIRMAN

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ABBREVIATIONS

8-OHdG	8-hydroxydeoxyguanosine
AAP	American Academy of Periodontology
AO	Antioxidant
CAL	Clinical Attachment Level
CEJ	Cemento -Enamel Junction
ChP	Chronic Periodontits
fMLP	Formyl-Methionyl-Leucyl-Phenylalanine
GBI	Gingival Bleeding Index
GCF	Gingival Crevicular Fliud
GPx	Glutathione Peroxidase
H_2O_2	Hydrogen Peroxide
MDA	Malondialdehyde
NSPT	Non-Surgical Periodontal Therapy
O_2	Oxygen
O_2^*	Superoxide Ion
PI	Plaque Index
PMN	Polymorphonuclear leukocytes
PPD	Pocket Probing Depth
ROS	Reactive Oxygen Species

SOD	SuperOxide Dismutase
SRP	Scaling and Root Planing
TAOC	Total Antioxidant Capacity
TNF	Tumor Necrosis Factor

INTRODUCTION

Smoking is a harmful habit which has its existence right from 16th century. Next to microorganisms, smoking has been considered as the strongest modifiable risk factor of periodontal disease with about 2 to 8 fold increase risk for attachment loss and bone loss[15]. Cigarette smoke not only has enormous free radicals but it also induces production of Reactive Oxygen Species (ROS) and depletion of antioxidants in tissues leading to oxidative stress and ultimately resulting in tissue damage.

Superoxide Dismutase (SOD) is a key antioxidant enzyme that specifically scavenges free radicals of oxygen. It is present in all tissues and body fluids including saliva [39]. Free radicals from cigarette smoke has a tendency to attack antioxidant enzyme (AO) than non-enzymatic form [12].

Chronic periodontitis is an inflammatory disease wherein one of the mechanisms of periodontal tissue destruction involves over production of ROS and depletion of AO like SOD. To overcome this imbalance in the equilibrium of ROS and AO a supplemental antioxidant can be a possible therapeutic modality.

Vitamin C is a scavenging and preventing AO which is capable of regenerating other antioxidants[56.] Many studies have shown association between smoking and vitamin C[50,63] and also periodontal disease and vitamin C[10,44,60]

Recently saliva has gained more attention as a diagnostic fluid. Whole saliva represents a pooled sample with contributions from all periodontal sites, analysis of biomarkers in saliva may provide an overall assessment of disease status as opposed to site – specific GCF analysis.[58] For this reason, the levels of several enzymes,

proteins and other constituents of saliva have been investigated for possible correlation with periodontal disease activity[24].

So far very few interventional studies have been conducted regarding the effect of non-surgical periodontal therapy supplemented with antioxidants on SOD levels.

Hence the aim of the present study is to estimate salivary SOD levels in smokers with chronic periodontitis and to ascertain the effectiveness of vitamin C supplementation following phase I therapy.

AIM

To assess the salivary dismutase levels in predicting the effectiveness of vitamin C supplementation in phase I therapy in smokers and non smokers with chronic periodontitis.

OBJECTIVES

1. To compare salivary SOD levels in smokers/nonsmokers with chronic periodontitis

2. To compare salivary SOD levels before and after phase I therapy in the same subjects.

3. To evaluate the effectiveness of vitamin C therapy as an adjunct to phase I therapy.

REVIEW OF LITERATURE

Periodontal diseases are inflammatory disorders that give rise to tissue injury and loss, as a result of the complex interactions between pathogenic bacteria and the host immune response.[20] It is likely that the role of reactive oxygen species (ROS) is common to both bacterial and host-mediated periodontal tissue damage. Recently the role of ROS in the pathogenesis of periodontitis has gained more attention.

SALIVA-DIAGNOSTIC MEDIUM:

Saliva is a secretion of the salivary glands, ensures stability in the oral cavity environment. "Whole saliva" is composed of saliva , gingival crevicular fluid contained in the dento-gingival sulcus, transudate, cell detritus, bacteria and food debris.[9] For periodontal diagnosis, use of saliva has been the subject of considerable research activity, and has proposed markers for disease including proteins of host origin (i.e. enzymes, immunoglobulins), host cells, hormones (cortisol), phenotypic markers (epithelial keratins), bacteria and bacterial products, volatile ions and compounds[47]**.** Enzymes present in saliva may be of host derived (cells in the salivary glands, PMNs, epithelial cells, and from GCF) or from microorganisms[19]**.** The correlation between its biomarkers and clinical features of periodontal disease has been evaluated for the following aspects of periodontitis – inflammation, collagen degradation and bone turnover.

For the past two decades, salivary diagnostic approaches are developed to monitor periodontal diseases[24], to assess caries risk[16], and to diagnose oral cancer[45]. Recently, due to emerging biotechnologies in salivary diagnostics, a large number of analytes in saliva are gradually unveiled, some of them represent biomarkers for different diseases including cancer, infections, etc[23]. The most challenging part of

salivary diagnostics is to identify disease markers and effectively translate the research efforts from the laboratory to the clinic.

Nieminen et al[54] stated that the enzyme activity of whole saliva likely reflects the periodontal disease severity, and salivary enzymes have the potential to assess periodontal inflammation and its response to periodontal treatment.

SUPER OXIDE ION - REACTIVE OXYGEN SPECIES (ROS):

In **1769 Fridovich** showed that superoxide ion was produced during an enzymatic oxidation. Superoxide is being formed by addition of an extra electron to the oxygen molecule.

Battino M et al[14] in **1999** made a remarkable expansion in medical and dental field concerned with free radicals, ROS and AO defense mechanism.

Sources of superoxide:

1.Exogenous sources:

Include heat, ultraviolet light, ozone, trauma, ultrasound, radiation, **smoking**, exhaust fumes, infection, excessive exercise, and therapeutic drugs[27]

2. Endogenous sources are primarily:

- Byproducts of metabolic oxidative pathways – electron leakage from mitochondrial electron transport systems leads to formation of superoxide[39]
- Functional generation by activated phagocytes such as PMNLs through "respiratory burst"[25] and cells like fibroblasts[52.]

ASSOCIATION OF BETWEEN SMOKING AND ROS IN PERIODONTITIS:

Smokers are exposed to over 40,000 chemicals from cigarette smoke and the combustion of tobacco creates almost 1 x 10 free radicals per cigarette [5x10 per puff]

Smoking disturbs neutrophil chemotaxis or phagocytosis[28] and stimulates oxidative burst[61]**,** increases the number of neutrophils found in systemic circulation**,** increase their formyl-methionyl-leucyl-phenylalanine (fMLP) receptors and leaving them "primed", which results in a two-fold increase in the release of elastase and superoxide ion in response to fMLP[59]. Neutrophils express functional receptors for several components and metabolites of tobacco like nicotine, cotinine (α3, β4 subtype of nicotine receptors) [61]

In animal and human studies, cigarette smoke has been found to affect both cell mediated and humoral immunity[40,68]. Nicotine inhibits antibody forming cell responses leading to immunosuppression that appears to be due to the effect of impairment of antigen mediated T cell signalling[68] Studies by **Hughes et al** in**. 1985**, showed leukocytosis in smokers[38]

An increased priming effect of TNF-α has also been shown in smokers with periodontitis accompanied by an increased generation of free radicals and up-regulated neutrophil function[34] **Gillespie et al.** [30] in **1987**, showed a potentiated release of superoxide ion from neutrophils in rats exposed to smoke.

There is convincing evidence that the antioxidant status of smokers is reduced. Cigarette smoke may result in an increased metabolic turnover, because of greater expenditure of antioxidant micronutrients from increased oxidative stress that is caused by the tobacco products, or smoking could decrease micronutrient absorption[7]**.** In contrary it has also been revealed that the antioxidant response may be overwhelmed as a protective mechanism counteracting the harmful effects of ROS.[72]

ASSOCIATION BETWEEN SMOKING AND PERIODONTITIS:

Stolenberg J.L. et al[70] in **1993** concluded that smoking is a stronger risk indicator for the mean posterior proximal probing depth 3.5mm.

Kinane D.F. et al[43]**.** in **1997** evaluated the effect of smoking on the outcome of periodontal therapy and stated that smoking has a negative effect on the prognosis of periodontal treatment mainly in persistent and deep pockets.

Bergstrom et al[15] in **2000** reported that heavy exposure to cigarette smoke was consistently associated with more severe condition than light exposure, signifying that the relationship between smoking and periodontal morbidity is dose-dependent and an avoidable risk factor for periodontal disease.

Nair P. et al[53] in **2003** emphasized on the effect of smoking in masking the signs and symptoms of inflammatory process and the reversible effect on quitting smoking.

Darby IB et al[26] in **2005** concluded that SRP was effective in reducing the severity of clinical parameters but an inferior improvement in smokers which was due to the systemic effects of smoking on the host response and in healing process.

Wan CP et al[73] in **2009** investigated the factors predicting responses of non-surgical periodontal treatment by multilevel multiple regressions and found that smokers showed less favourable probing depth reduction at deep sites.

ANTIOXIDANT DEFENSE SYSTEMS

To counteract the harmful effects of ROS, a number of antioxidant defense mechanism exists. **Halliwell**[36] in **1994** defined antioxidant as "any substance that when present at low concentrations compared to those of an oxidisable substrate, significantly delays or inhibits the oxidation of the substrate"

Antioxidants (AO's) are classified according to their mode of action as follows:

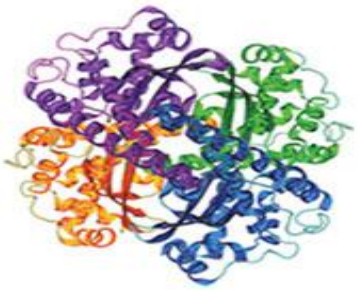
1. Preventive antioxidants: Catalase, SOD, Carotenoids, Transferrin, Ceruloplasmin,
2. Radical scanvenging: Ubiquionol, Vitamin A, Vitamin E, Carotenoid, Uric acid, ascorbic acid, albumin, Bilirubin
3. Repair and de-novo enzymes: DNA repair enzymes, protease, transferase, lipase[21]

SUPEROXIDE DISMUTASE ENZYME

Superoxide dismutase (SOD) is a metalloenzyme and its main active centre is occupied by copper and zinc, manganese or iron. SOD plays an important role in the protection of all aerobic lives, including man, against oxygen toxicity and the free radicals derived from oxygen.

Joe M. McCord[39] in **1969** analysed the abundance of SOD activity in animal tissue and suggested that SOD might play a significant role in protecting the organisms from the damaging effects of superoxide radical. **McCord JM**[48] in **1978** stated that "SOD exerts an anti-inflammatory action that may be useful in managing inflammation".

Figure 1 : Structure of Superoxide Dismutase



SOD catalyses the conversion of $O_2^{\bullet-}$ to H_2O_2 via a dismutation reaction Superoxide dismutase uses one $O_2^{\bullet-}$ radical to oxidize another.

$$2O_2^{*} + O_2^{*} + 2H^{+} \rightarrow H_2O_2 + O_2$$

There are three forms of superoxide dismutase in mammalian tissues, each with a specific sub-cellular location and different tissue distribution.

1. Copper zinc superoxide dismutase (Cu/ZnSOD): It is found in the cytoplasm. In humans, Cu/Zn SOD is assumed to play a major role in the first line of AO defense.

2. Manganese superoxide dismutase (Mn-SOD): It is found in mitochondria.

3. Extracellular superoxide dismutase (EC-SOD): It was described by **Marklund in 1982**. It is synthesized by few cell types, like fibroblasts and endothelial cells, and expressed on the cell surface where it is bound to heparin sulphates.

Sculley and Langley-Evans[64] in **2003** examined the antioxidant conditions in the saliva and reported that gingivitis and periodontitis were associated with a decreased salivary antioxidant level and increased oxidative injury. They also reported that low concentrations of GCF antioxidants increase the neutrophil-induced injuries in the gingiva.

Akalin FA et al[5] in **2005** compared SOD activities in gingiva and GCF from patients with ChP and periodontally healthy controls. In ChP, SOD activity appears to increase in gingiva, possibly as a result of a higher necessity for SOD activity and protection in gingiva in ChP, while not changed significantly in GCF, which suggest a weak SOD activity in GCF.

Baltacioglu et al[13] in **2006** compared the serum and GCF total antioxidant capacity (TAOC) and SOD concentrations in post-menopausal and pre menopausal

women with ChP. Results showed that serum and GCF TAOC and SOD concentration were significantly lower in menopause and ChP subjects.

Akalin FA[4] in **2009** compared serum and gingival crevicular fluid (GCF) total AO capacity (TAOC) and SOD concentrations in pregnant patients with ChP with non-pregnant patients. TAOC and SOD levels were lowest in the pregnant patients with ChP group in the third trimester ($P < 0.05$).

Canakci et al[18] in **2009** evaluated 8-hydroxydeoxyguanosine, Malondialdehyde levels, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in whole saliva of patients with chronic periodontitis. Their findings showed a higher 8-OHdG and MDA levels in saliva and lower salivary SOD and GPx activities in ChP patients compared to the periodontally healthy controls.

Kim et al[42] in **2010** compared the total antioxidant status (TAS) and superoxide dismutase (SOD) activity in the saliva of ChP patients before and after scaling and root planing. The SOD activity in the test subjects decreased immediately after SRP until 1 month. At 3 months after SRP, the SOD activity had increased.

ASSOCIATION BETWEEN SMOKING AND SOD:

Gururaj et al[33] in **2004** estimated the antioxidants in oral exfoliated cells. Their findings showed that both the glutathione reductase and SOD enzyme activities in the exfoliated cells were significantly higher in the non smoker group as compared to smokers. The reduction in the level of AO in smokers may be due to tobacco smoke induced free radical liberation. This indicates that in smokers, the cells are under oxidative stress and damage.

Kanehira et al[41] in **2006** measured the salivary SOD level of smokers and concluded that measurement of SOD in saliva might be used to estimate the level of oxidative stress on smoking habits

Garg et al[29] in **2006** evaluated the association between smoking and periodontal damage in terms of free radicals and antioxidant levels. They concluded that SOD levels were higher in non-smokers with ChP than smokers with ChP both in tissue and blood. They concluded that smoking increases free radicals level in periodontal tissues, which in turn may be reason for the destruction seen in periodontal disease.

Buduneli N et al[17] in **2006** evaluated possible effects of smoking and gingival inflammation on antioxidants in saliva in gingivitis patients. They concluded neither smoking nor gingival inflammation affected the salivary antioxidant capacity in systemically healthy gingivitis patients.

Agnihotri R et al[2] in **2009** assessed the influence of smoking on the periodontium by estimating the levels of SOD in light and heavy smokers with periodontitis in saliva and GCF. The findings showed that the SOD level in GCF and saliva of smokers were decreased compared to periodontally healthy controls. Their study also showed a progressive reduction in SOD levels in healthy controls to light smokers to heavy smokers, signifying that increased oxidative stress and nicotine in smokers had resulted in the depletion of antioxidant enzymes

Pasupathi et al[57] in **2009** determined the effect of cigarette smoking on changes in lipid profile, lipid peroxidation and antioxidant status in smokers. Biochemical parameters such as cardiac markers, lipid profile, and antioxidants like SOD, catalase, glutathione peroxidase, vitamin A, C and E were also measured. The findings showed that there was a significant increase in levels of cardiac markers, but a huge depletion

of antioxidants in smokers as compared to non smokers. The researchers concluded that the atherogenic effects of smoking are mediated partly by free radical damage to lipids and breakdown of antioxidant status in cigarette smoking.

Hamid-reza Abdolsamadi et al[37] in **2011** compared the salivary antioxidant levels between healthy smoking and non-smoking men. The mean levels of salivary superoxide dismutase and glutathione peroxidase were significantly lower in smokers than non-smokers. Measurement of antioxidant agents in saliva might be useful for estimating the level of oxidative stress caused by smoking.

VITAMIN C - AN ANTIOXIDANT:

Vitamin C is a chain-breaking antioxidant that has anticarcinogenic and immunomodulatory actions. This antioxidant has to be supplemented only through diet as human cells cannot generate vitamin C.

In **1747 James Lind** conducted experiments aboard the ship ‘‘the Salisbury’’ in which he cured scurvy with lemons and oranges[71]**Meyle and Kapitza**[49] in **1990** reported that levels of vitamin C in GCF is 3 times higher than plasma. **Sagan et al**[62] in **2005** suggested that dietary vitamin C enters the mitochondria of the cell and protects against oxidative injury.

Possible etiologic relationships between ascorbic acid and periodontal disease.

1. Ascorbic acid deficiency may lead to
    1. Impairment in regeneration and repair of periodontal tissue due to impairment in the collagen metabolism.
    2. Interferes with bone formation, leading to loss of periodontal bone.
    3. Increases the permeability of the oral mucosa to endotoxin and inulin and of normal human crevicular epithelium to dextran.

4. May interfere with the ecologic equilibrium of bacteria in plaque and thus increase its pathogenicity

2. Increasing levels of vitamin C enhance both the chemotactic and migratory action of leukocytes (neutrophils) without influencing their phagocytic activity

3. An optimal level of vitamin C is apparently required to maintain the integrity of the periodontal microvasculature; and the vascular response to bacterial irritation and wound healing.[19]

Chapple [21] in **2007** summarized its role as an antioxidant:

• Scavenging water-soluble peroxyl radicals.

• Scavenging superoxide and perhydroxyl radicals.

• Prevention of damage mediated by hydroxyl radicals on uric acid.

• Scavenger of hypochlorous acid.

• Preventing Fenton reactions.

• Scavenger of singlet oxygen and hydroxyl radicals.

• Re-forms a-tocopherol from its radical.

• Protects against ROS-release from cigarette smoke.

Association between vitamin C and smoking

Schectman, et al[63] in **1989** conducted a study to define the independent relationship between smoking and vitamin C status. The association of smoking with serum vitamin C levels was analyzed. The author concluded an inverse relation exists between smoking and dietary vitamin C intake. This confirms that smokers have lower concentrations of vitamin C in serum than non-smokers.

Mieko Nishida et al[50] in **2000** evaluated the role of dietary vitamin C as a risk factor for periodontal disease utilizing Third National Health and Nutrition

Examination Survey. The authors concluded that dietary intake of vitamin C showed a weak, but a significant, relationship to periodontal disease in former and current smokers as measured by clinical attachment level.

Greabu et al[32] in **2007** studied the effect of vitamin C on salivary antioxidants and showed that cigarette smoke decreases uric acid level in saliva. Addition of 10mg/dl vitamin C to saliva was not able to restore the original uric acid level but it significantly increased uric acid level. This implies, vitamin C has a protective effect on uric acid level in saliva. They suggested that an adequate amount of antioxidant intake may help smokers to overcome cigarette smoke-induced oxidative damage.

Bakhtiari Sedighe et al[12] in **2011** performed a study to elucidate the effect of vitamin C on salivary superoxide dismutase (SOD) activity in smokers and concluded that vitamin C does not improve SOD activity significantly. It is possible that smoke–induced oxidative stress might decrease after vitamin C intake.

ROLE OF VITAMIN C IN PERIODONTAL THERAPY:

Leggot et al[44] in **1986** conducted a study to determine whether systemic levels of vitamin C influence periodontal health, by measuring PI gingival health and PD in healthy subjects who were provided controlled periods of ascorbic acid depletion and supplementation for three months. No changes in PI or PD were found during any of the periods of depletion or supplementation. But gingival inflammation measurements were directly related to the ascorbic acid status. The finding suggest that vitamin C may influence early stages of gingivitis, principally crevicular bleeding.

Pussinen et al[60] **2003** in studied the relationship between periodontitis and the concentration of ascorbic acid in serum. They reported that patients with periodontitis, who were infected by P. gingivalis, showed a lower ascorbic acid level in the serum than those not infected.

Staudte H et al[69] in **2005** examined the plasma vitamin C levels and inflammatory parameters in periodontitis patients before and after taking grape fruit. The intake of grape fruit resulted in an increase in plasma vitamin C levels and improved sulcus bleeding scores.

Amaliya et al[10] in **2007** conducted a study, comparing the relationship between vitamin C and the severity of periodontitis. They observed negative correlation between plasma vitamin C level and periodontal attachment loss ($p<0.05$). This suggests that vitamin C deficiency may contribute to the periodontal breakdown.

Ali E et al[8] in **2010** investigated plasma total antioxidant capacity in patients with ChP and assessed the effects of vitamin C as an adjunct to phase I therapy. The author observed that ChP was significantly associated with lower levels of plasma TAOC. Phase I therapy had reduced the oxidative stress during the periodontal inflammation. But no improvement with vitamin C supplementation was noted. However, the use of adjunctive vitamin C still needs further investigation.

Abdul Samed Aziz et al[1] in **2012** evaluated and compared, following parameters total antioxidant capacity (TAOC), RBC-SOD, GPx, Vitamin C, Malondialdehyde (MDA) and C-reactive protein (CRP) between controls and CP. Obtained results suggest that oxidative stress is induced in chronic periodontitis. As compared to periodontally healthy controls the levels of CRP, MDA and RBC-SOD were significantly higher ($p<0.001$) and those of TAOC, GPx and vitamin C were significantly lower ($p<0.001$) in patients with chronic periodontitis

Akmans et al[6] in **2012** investigated the role of Alpha-Lipoic Acid (ALA) and Vitamin-C in the treatment of alveolar bone resorption in periodontal diseases. The author evidenced that ALA and Vitamin-C treatment provided beneficial effects on inhibition of periodontal tissue destruction and alveolar bone resorption in rats.

MATERIALS AND METHODS

Subject Selection:

The study population consisted of 70 subjects who attended the Outpatient Section of the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai, India. Ethical clearance was obtained from the Institution's Ethical Committee.

Inclusion Criteria

- Minimum of 20 teeth present
- 35 – 60 years of age,
- Gender – Males

Exclusion criteria included:

- Patients with underlying systemic diseases
- Patient who have received medication (antibiotics, anti inflammatory, steroids, anxiolytics) for past 4 to 6 months.
- History of vitamin supplements for the past 3 months.
- Patients with other oral habits (tobacco and pan chewing)
- Absence of any lesions in the oral cavity, and
- Patients who had undergone periodontal treatment in past 6 months.

Group I: Control Group:

Sample size n = 10

- No sites with Probing Depth >3 mm
- Less than 20% of sites exhibiting gingival bleeding.

Group II: Study Group- non smokers:

Sample size n = 30

The diagnosis of Chronic Periodontitis was established on the basis of clinical and radiographic criteria (bone loss) according to the AAP 1999 classification system for periodontal diseases and conditions[11].

- A minimum of six teeth with at least one site each with Probing Depth ≥5mm and Clinical attachment level ≥1 mm, and
- Presence of Bleeding on Probing.

Group II was further divided into:

- **Group II A:** SRP only (n = 15)

  Group IIA_1: After therapy
- **Group II B:** SRP with vitamin C supplementation (n = 15)

  Group II B_1: After therapy

Group III: Study Group- smokers :

Sample size n = 30

The diagnosis of Chronic Periodontitis was established on the basis of clinical and radiographic criteria (bone loss) according to the AAP 1999 classification system for periodontal diseases and conditions[11].

- A minimum of six teeth with at least one site each with Probing Depth ≥5mm and Clinical attachment level ≥1mm, and
- Presence of Bleeding on Probing.
- Smokers

Group III was further divided into:

Group III A: SRP only (n = 15)

Group III A_1: After therapy

Group II B: SRP with vitamin C supplementation (n = 15)

Group III B_1: After therapy

In addition, all subjects should meet the following criteria:

- Should have controlled oral hygiene during periodontal treatment,
- Agreement to participate in the postoperative control program.

STUDY PROTOCOL

1. Medical History and Informed Consent
2. Complete Periodontal Examination using clinical parameters namely Gingival Bleeding Index, Plaque Index, Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL)
3. Radiographic evaluation of generalized chronic periodontitis
4. Collection of saliva samples
5. Appropriate treatment performed for Group II and Group III patients
6. Re-evaluation of Group II and Group III patients using clinical parameters namely, Gingival Bleeding Index, Probing Pocket Depth and Clinical Attachment Level.
7. Collection of saliva samples in Group II and Group III patients 1 month after therapy

8. Estimation of SOD in saliva samples by spectrophotometric analysis.

Subjects for the study were selected randomly, with no discrimination on the basis of age, caste, religion or socioeconomic status between control and study groups. Following selection of subjects, written informed consent (**Annexure 2 & 3**), which was approved by the Institute's Ethical Committee, was obtained from all the subjects selected for the study after explaining the study procedure. Examination was preceded by a thorough medical and dental history of the subjects (**Annexure 4**). Each subject underwent full-mouth periodontal probing and for radiographic evaluation orthopantomogram was taken. Radiographic bone loss was recorded dichotomously (as presence or absence) to differentiate patients with periodontitis from other groups. Then about 2ml of unstimulated whole saliva was collected from the subjects for spectrophotometric analysis.

PERIODONTAL EXAMINATION:

CLINICAL PARAMETERS

The following clinical parameters were evaluated for all patients:

1. Gingival bleeding index – ***Ainamo and Bay 1975***.
2. Plaque index – ***Silness and Loe 1964***
3. Probing Pocket Depth in mm (PPD) – ***Carranza 10th ed***
4. Clinical attachment level in mm (CAL) – ***Carranza 10th ed***

Gingival Bleeding Index (*Ainamo and Bay 1975*)[3]

Starting distobuccally, the probe was inserted slightly into the sulcus and run to the buccal and mesial surfaces of every tooth at an angle of about 45°. This was repeated for all teeth present. Probing was similarly carried out at palatal/lingual sites. Any gingival units that exhibited bleeding were recorded. The total number of bleeding sites per tooth was thus recorded for every tooth except the third molar.

Criteria for Scoring

Positive score (+) - Presence of bleeding within 10 seconds

Negative score (-) - Absence of bleeding

$$\% \text{ of bleeding sites} = \frac{\text{Total number of positive score}}{\text{Total number of surfaces of all teeth}} \times 100$$

Plaque Index *(Silness and Loe 1964*) [67]

All teeth were examined at 4 surfaces each (disto-facial, facial, mesio-facial, lingual/palatal) and were scored as follows.

Criteria for Scoring:

Score 0 No plaque

Score 1 Plaque not visible to the naked eye, detected by explorer

Score 2 Thin to moderate accumulation of soft deposits within the gingival

pocket or on tooth, visible to the naked eye

Score 3 Abundance of soft matter within gingival pocket or on tooth surface and margin, inter-dental area stuffed with soft debris

Calculation :

Plaque index per tooth = Total score/4

Plaque index per individual = Total PI per tooth / Total number of teeth examined

Interpretation:

Score 0 – Excellent oral hygiene

0.1 to 0.9 – Good oral hygiene

1.0 to 1.9 – Fair oral hygiene

2.0 to 3.0 - Poor oral hygiene

Probing Pocket Depth (PPD)(In mm)*(Carranza 10th ed)*[19]

Probing Pocket Depth was measured from the gingival margin to the base of the pocket in millimeters using Williams Periodontal Probe. The probe was walked within the gingival sulcus along the circumference of the tooth. Six measurements were made per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual).

Clinical Attachment Level (CAL) *(Carranza 10th ed)*[19]

Clinical Attachment Level was measured from the Cemento – Enamel Junction (CEJ) to the base of the pocket using Williams Periodontal Probe.

- When the gingival margin was located on the anatomic crown, the level of the attachment was determined by subtracting from the probing pocket depth, the distance from the gingival margin to the CEJ. If both were the same, the loss of attachment was calculated to be zero.

- When the gingival margin coincided with the CEJ, the loss of attachment was calculated as equaling the probing pocket depth.

- When the gingival margin was located apical to the CEJ, the loss of attachment was greater than the probing pocket depth and therefore the distance between the CEJ and the gingival margin were added to the PPD.

Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth – total of six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual).

ARMAMENTARIUM

For clinical examination

Mouth mirror

Williams calibrated periodontal probe

Curved explorer

Dental tweezers

Kidney tray

Cotton roll

Sterilized disposable gloves

Disposable facemask

For collection of saliva sample

Vaccutainer .

Sterile cotton.

For saliva storage :

-20^0 C freezer

For SRP :

Mouth mirror

Explorer

Scalers and Curettes

Kidney Tray

Cotton Rolls

Disposable Gloves

Disposable Facemask

Disposable Headcap

Disposable syringe

Local Anaesthetic solution

For Enzymatic Assay

Diagnostic Reagent kits

Spectrophotometer

Distilled Water

Sterile test tubes

Micropipettes

Syringes

Saliva Sample Collection

Saliva sample was collected at:

- **Baseline**
- **4 weeks post treatment**

Samples of unstimulated, whole saliva was taken before and after treatment in empty stomach. Immediately after rinsing with water, 2ml Saliva of the patient was collected in vaccutainer. The saliva sample was stored at -20^0 C in bio-chemical laboratory and the activity of the superoxide dismutase salivary enzyme was determined spectrophotometrically.

After saliva sample collection appropriate treatment was performed to each group.

Group I:

No treatment required and kept under maintenance.

Group IIA and IIIA:

Nonsurgical periodontal therapy consisting of scaling and root debridement done and oral hygiene instructions given.

Group IIB and IIIB:

Nonsurgical periodontal therapy consisting of scaling and root debridement done. Oral hygiene instructions given. Vitamin C supplement 500mg OD for 4 weeks given.

Laboratory Procedure for Assessment of Superoxide Dismutase Enzyme Activity

Principle:

Superoxide dismutase role is to accelerate the dismutation of the toxic superoxide ion, produced during oxidative energy process, to hydrogen peroxide and molecular oxygen. The kit used for study employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye.

The superoxide dismutase function is then measured by the degree of inhibition of the reaction.

$$Xanthine \xrightarrow{xanthine\ oxidase} Uric\ acid + O_2^-$$

$$INT \xrightarrow{O_2^-} Formazan\ dye.$$

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

Reagent preparation:

1.Mixed Substrate(R1):

Reconstitute the contents of one vial of Mixed Substrate R1 with 20ml of Buffer R2.

2.Buffer (R2):

Content is ready to use.

3.Xanthine oxidase (R3):

Reconstitute the contents of one vial of R3with 10ml of distilled water.

4. Standards (R4):

Reconstitute the contents of one vial of R4 with 10ml of distilled water. Subsequent dilutions of this standard must be with sample diluent (R5). The following dilutions are made of standard S6 to produce a standard curve.

	Vol of standard solution	**Vol of sample diluent**
S6	Undiluted standard	
S5	5ml of S6	5ml
S4	5ml of S5	5ml
S3	5ml of S4	5ml
S2	3ml of S3	5ml

S1 = Sample Diluent

5.Sample Diluent: Content ready to use.

Sample :

0.5ml of distilled water, 0.25ml ethanol and 0.15ml chloroform were added to 0.5 ml collected saliva, mixed in a vortex machine. Then 0.1ml of distilled water was added and the solution was centrifuged at 2000rpm for 15min. The supernatant was diluted 1 in 100 times with SOD sample diluents to give diluted sample.

Manual procedure:

Wave length	Temperature	Cuvette	Measurement
505nm	**37^0C**	**1cm light path**	**Against air**

Pipette into test tubes as follows:

	Sample diluent	**Standards S2 –S6**	**Diluted Sample**
Diluted Sample			**50µl**
Standard		**50µl**	
Sample diluent	**50µl**		
Mixed Substrate	**1.7ml**	**1.7ml**	**1.7ml**
Mix well, then add:			
Xanthine Oxidase	**250µl**	**250µl**	**250µl**

The mix was incubated for 30s at 37^{0}C, then first reading was taken. Readings were taken again after 1,2 and 3 minutes.

To determine the mean absorbance change per min (∆A) the following calculation was used.

Calculation:

To calculate the SOD value = $\frac{A2-A1}{3}$ = ∆ A/min of standard or sample

For standard:

100 – $\frac{Astd\ /\min\ x100}{As1/min}$ = % inhibition

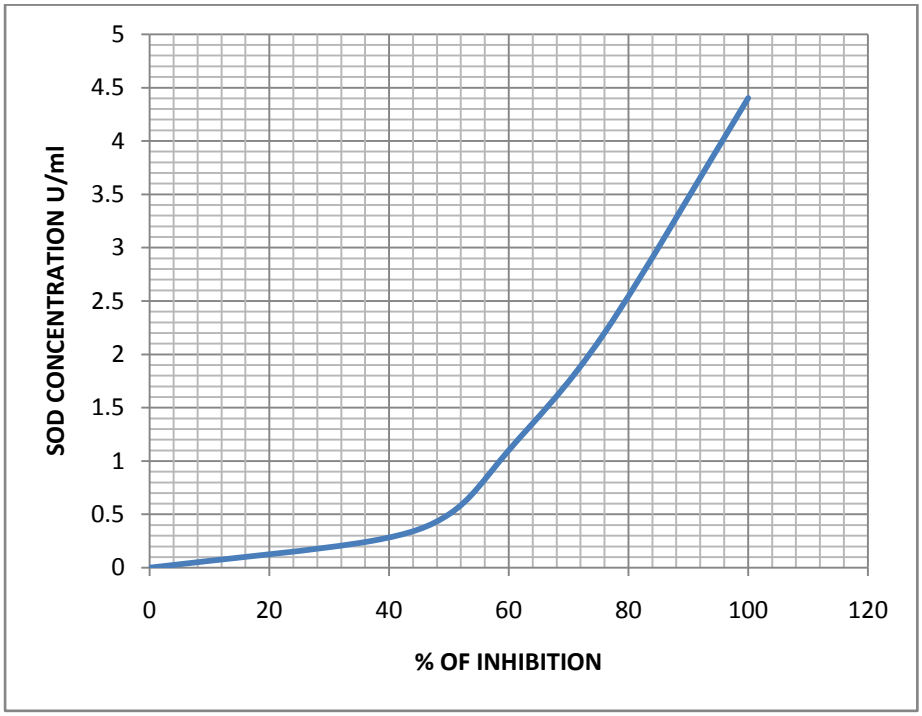
For sample

100 – $\frac{Asample\ /\min\ x100}{As1/min}$ = % inhibition

Plot percentage inhibition for each standard against standard conc. in SOD units/ml

Percentage inhibition of sample was used to obtain units of SOD from standard curve.

Figure 2: Standard curve.



SOD units/ml of whole saliva = SOD units/ml from standard curve X dilution factor

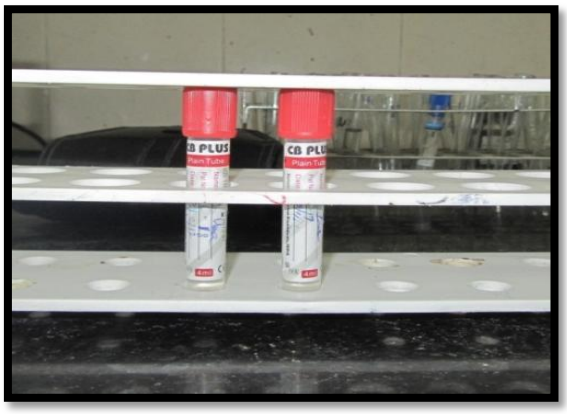
Photograph 1 : Healthy Controls

Photograph 2: Generalized Chronic Periodontitis

Photograph 3: Orthopantamogram for Chronic Periodontitis Group

Photograph 4: Armamentarium for Clinical Examination & Sample Collection

Photograph 5: Clinical Examination using Williams periodontal probe



Photograph 6: Salivary Samples

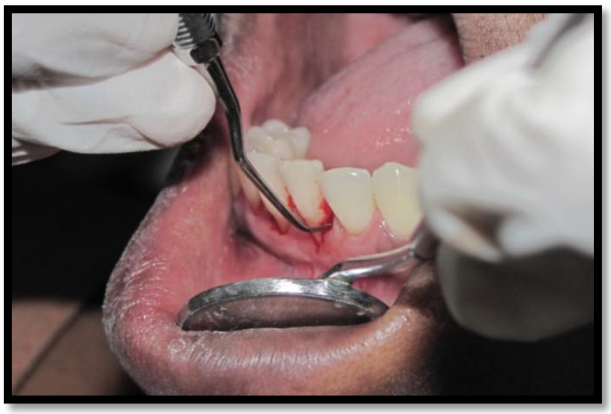
Photograph 7: Centrifuge machine

Photograph 8: Reagents for SOD Enzyme Analysis

Photograph 9: Spectrophotometer

Photograph 10: Micropipette

Photograph 11: Armamentarium for phase I therapy



Photograph 12: Phase I Therapy

Photograph 13: Before Phase I Therapy

Photograph 14: After Phase I Therapy

STATISTICAL ANALYSIS

The statistical package SPSS V16 (Statistical Package for Social Science, Version 16) was used for statistical analysis.

PAIRED t TEST:

For a comparison of more than one pair of split sample test results, the t-test for paired measurements can be used.

The formula of the *t*-test for paired measurements is:

$$t = \frac{\left\| \left| \overline{X}_d \right| - \left| X_O \right| \right\|}{\frac{s_d}{\sqrt{n}}}$$

Where: $\overline{X_d}$ = Average of the differences between the individual split sample test results

X_O = The value of the expected difference between split sample tests. In most cases $X_0=0$, as there is no expected difference between contractor and agency tests. However, X_0 could reflect an expected correlation value between testing entities.

s_d = Standard deviation of the differences between the split sample test results

n = Number of split samples

INDEPENDENT t TEST:

To compare the statistical significance of a possible difference between the means of two groups on some independent variable and the two groups are **independent** of one another. The formula for the independent t-test is

$$t = \frac{X_1 - X_2}{\sqrt{\left(\frac{SS_1 + SS_2}{n_1 + n_2 - 2}\right)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}},$$

Where,

X_1 is the mean for group 1,

X_2 is the mean for group 2,

SS_1 is the sum of squares for group 1,

SS_2 is the sum of squares for group 2,

$\mathbf{n_1}$ is the number of subjects in group 1, and

$\mathbf{n_2}$ is the number of subjects in group 2.

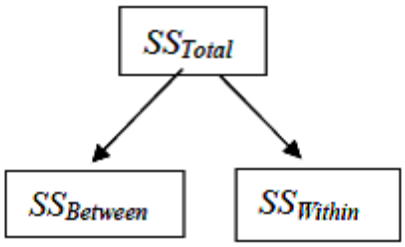
One-Way ANOVA:

Computational Formulas for ANOVA

Null Hypothesis states that H0: μ1 = μ2 = μ3 = . . . = μa

ANOVA analyzes sample variances to draw inferences about population means.

Sample variances can always be calculated as *SS/df* and these sample variances are called mean squares (*MS*).



$$SS_{Total} = \sum X^2 - \frac{\left(\sum X\right)^2}{N} \qquad df_{Total} = N - 1$$

$$SS_{Between} = \frac{\left(\sum X_1\right)^2}{n_1} + \frac{\left(\sum X_2\right)^2}{n_2} + \ldots + \frac{\left(\sum X_a\right)^2}{n_a} - \frac{\left(\sum X\right)^2}{N} \qquad df_{Between} = a - 1$$

$$SS_{Within} = SS_{Total} - SS_{Between} \qquad df_{Within} = N - a$$

$$s^2 = \frac{SS}{df} = MS \qquad F = \frac{MS_{Between}}{MS_{Within}}$$

Where,

SS – Sum of Squares

MS – Mean Squares

N = total no of observations in the experiment

a = No of groups

n_1 = no of observations in group 1, etc.

F = observed Variance Ratio

tCrit is the critical value from a *t*-table using the *df* of the error term from the ANOVA table. F > *tCrit at 5%value means that the difference between the groups is significant at the 5% value.*

P value:

The P value or calculated probability is the estimated probability of rejecting the null hypothesis (H0) of a study question when that hypothesis is true. Differences between the two populations were considered significant when $p < 0.05$.

PEARSON CORRELATION:

Pearson correlation (Bivariate) was used to analyze the strength of association between the investigated variables. The correlation coefficient (r) was interpreted as follows.

0.0 - 0.1 - Trivial,

0.1 - 0.3 - Low

0.3 - 0.5 - Moderate

0.5 - 0.7 – High

0.7 - 0.9 - Very high

0.9 - 1 - Nearly perfect

RESULTS

In the present study, a total of 70 male subjects were included, among them 10 were periodontally healthy controls (Group I), remaining 60 subjects with chronic periodontitis were divided into two groups, Group II non-smokers with chronic periodontitis and Group III smokers with chronic periodontitis consisting of 30 subjects in each group. Group II and Group III were further divided into Group IIA ; Group IIB and Group IIIA ; Group IIIB respectively, with 15 members each, based on the interventional treatment provided. Subjects treated with SRP alone were designated as Group IIA and Group IIIA and those treated with SRP with an adjunct vitamin C were designated as Group IIB and Group IIIB. Subsequently the post-treatment Groups were designated as A_1 and B_1 for each respective Group.

Table 1, 2, 3, 4 and 5 represents the master chart for Group I,IIA,IIB,IIIA and IIIB respectively. The mean age was 42.83 (Group I- 41.5±5.77, Group II- 44.3±6.41 and Group III -42.96±6.44) *(table 6; fig.3).*

CLINICAL FINDINGS:

The clinical parameters used were plaque index (PI), gingival bleeding index (GBI), pocket probing depth (PPD) and clinical attachment level (CAL).

The plaque index score was significantly higher in Group II (2.20±0.34) and Group III (2.29±0.37) when compared to Group I (0.25±0.23) *(table 7 ; fig.4).* PI score shows negative correlation with SOD level in all Groups but shows positive correlation in Group III, though the correlation was not significant. *(table 20).*

The GBI was significantly higher in Group II (86.55±6.73) as compared to Group III (29.613±10.86) and both these Groups scores were significantly higher as

compared to Group I (15.37±7.81) with a p value >0.01.*(table 8; fig.5).*The post-treatment values of Group IIA_1 (15.70±3.2) Group IIB_1 (13.15±2.55), Group III A_1 (12.65±2.36) and Group III B_1 (15.15±2.92) were significantly reduced as compared to the pre-treatment value of Group II A (83.94±4.47), Group II B (84.71±8.18), Group III A (27.70±10.5) and Group III B (31.52±11.2) respectively *(table 10, 11, 12 & 13; fig.5).*The post-treatment values were almost to the level of Group I (15.37+7.81) GBI score *(table 16, 17, 18 & 19).*The GBI score shows negative correlation with SOD level of all Groups, but shows positive correlation in Group II and Group IIB_1, though all were not significant with p value >0.05. *(table 20&21; fig. 9,12,15,18,21,24,27,30&33).*

The PD was significantly higher in Group II (4.87±0.81) and Group III (4.82±0.76) as compared to Group I (1.64±0.29) with a p value <0.01. *(table 8, fig.6).*There was no significant difference in PD between Group II (4.87±0.81) and Group III (4.82±0.76) with a p value 0.798 *(table 9; fig. 6).*The post treatment values of Group IIA_1 (3.26±0.33) Group IIB_1(3.38±0.52), Group III A_1 (3.29±0.513) and Group III B_1 (3.412±0.37) were significantly reduced as compared to the pre-treatment value of Group IIA (4.96±0.75), Group IIB (4.79±0.87), Group III A (4.526±0.73) and Group IIIB (5.124±0.63) respectively *(table 10,11,12&13; fig.6).* There was negative correlation between PD and SOD level of all Groups, but shows positive correlation in Group II**.** The correlation was significant only in Group $IIIA_1$. *(table 20&21; fig. 10,13,16,19,22,25,28,31&34).*

No loss in CAL was observed in Group I. There was no significant difference in CAL between Group II(5.10±0.742) and Group III(5.150±0.75) with a p value 0.802.*(table 9, fig.7).* The post treatment values of Group IIA_1 (3.44±0.47), Group IIB_1 (3.62±0.59), Group III A_1 (3.55±0.55) and Group $IIIB_1$ (3.82±0.54) were

significantly reduced as compared to the pre-treatment value of Group IIA (5.14±0.54), Group IIB (5.06±0.92), Group III A (4.85±0.61) and Group IIIB (5.46±0.76) respectively *(table 10, 11, 12 and 13; fig.7).* There was negative correlation between CAL and SOD level of all Groups, but shows positive correlation in Group II and Group $IIIB_1$. The correlation was significant only in Group $IIIA_1$ *(table 20&21; fig. 11,14,17,20,23,26,29,32&35)*..

LABORATORY FINDINGS OF SALIVARY SUPEROXIDE DISMUTASE LEVEL

The baseline salivary SOD level was significantly less in Group II (25±3.99) and Group III (13.75±2.84) compared to control Group (149.2± 10.2) , p value <0.01 *(table 8; fig.8).* The mean SOD of Group III (13.75±2.84) was significantly less as compared to Group II (25±3.99) with a p value <0.01 *(table.9; fig.8)* **.**

There was no significant difference in the pre treatment level of SOD in Group II A (25.5±4.03) and Group III A (13.33±2.43) to the post-treatment level in Group II A_1 (25.67±3.94) and Group $IIIA_1$ (13.83±2.96) respectively *(table 10,12; fig.8).* SOD level significantly increased in post-treatment of Group II B_1 (67±12.47) and Group III B_1 (30.37±7.49) as compared to pre-treatment of Group II B (24.5±4.03) and Group III B (14.17±3.23) respectively *(table 11,13;fig.8).*

There was a significant difference in the post treatment level of SOD of Group IIA_1 (25.67±3.94) as compared to Group II B_1 (67±12.47) with a p value <0.01*(table 14).*There was a significant difference in the post treatment level of SOD between Group III A_1 (13.83±2.96) and Group III B_1 (30.37±7.49) with p value <0.01 *(table15).* The post treatment level of SOD had increased in GroupIIB_1 (67±12.47) and Group III B_1 (30.37±7.49) but not to the level of periodontally healthy controls (149.2± 10.2) *(table17,19).*

Table 1: MASTER CHART 1 GROUP I (CONTROL)

SNO	AGE	PI	GBI (%)	PPD(mm)	CAL(mm)	SOD (U/ml)
1	35	0.48	14.3	1.85	0	135
2	47	0.74	28	1.94	0	147.5
3	38	0.26	16.7	2.1	0	160
4	40	0.4	22.3	1.86	0	152.5
5	40	0.11	11	1.45	0	137.5
6	55	0.19	10	1.6	0	142.5
7	*43*	0.09	12	1.42	0	155
8	37	0.05	18	1.42	0	140
9	39	0.02	0	1.14	0	165
10	41	0.12	21.4	1.65	0	157.5

Table 2: MASTER CHART 2 GROUP IIA (NON SMOKERS- SRP ALONE)

S.NO	AGE	PI1	GBI1 (%)	PPD1 (mm)	CAL1 (mm)	SOD1 (U/ml)	GBI2 (%)	PPD2 (mm)	CAL2 (mm)	SOD2 (U/ml)
1	39	2.6	85.2	5.53	5.53	35	10.94	3.05	3.05	35
2	45	2.03	89	4.9	4.9	30	18.3	3.29	3.29	30
3	50	1.84	89	5.43	5.52	22.5	15.6	3.17	3.22	25
4	38	1.85	81.9	3.03	4.79	27.5	15.83	3.24	3.24	27.5
5	39	2.13	92.18	4.95	5.06	25	15.62	3.11	3.33	25
6	35	2.47	89.1	4.35	4.49	27.5	13.28	3.14	3.24	27.5
7	44	2.25	95	4.4	4.4	22.5	17.6	3.21	3.21	22.5
8	48	1.91	94.2	4.5	4.6	25	19.2	3.24	3.24	25
9	54	1.85	84.25	5.2	5.2	25	20.4	4.29	4.69	25
10	40	1.69	83.3	5.5	5.7	27.5	13.5	3.53	4.08	27.5
11	45	2.69	89.13	5.67	5.67	22.5	7.6	3.44	4.12	22.5
12	50	2.29	84.4	4.59	4.59	17.5	18	3.15	3.15	17.5
13	53	1.85	83.2	5.14	5.14	25	16.6	3.34	3.38	25
14	52	2.44	94.53	4.91	5.12	27.5	17.2	2.9	3	27.5
15	43	1.88	91.53	6.33	6.33	22.5	15.89	2.88	3.38	22.5

Table 3: MASTER CHART 3 GROUP IIB (NON SMOKERS- SRP+ VITAMIN C)

S. NO	AGE	PI1	GBI1 (%)	PPD1 (mm)	CAL1 (mm)	SOD1 U/ml	GBI2 (%)	PPD2 (mm)	CAL2 (mm)	SOD2 U/ml
1	38	2	75	6.21	6.74	22.5	13.6	3.88	4.17	80
2	40	2.11	90	6.38	6.71	25	12	3.96	4.23	52.5
3	35	2	89.6	4.89	5.1	22.5	10.4	3.12	3.4	57.5
4	38	2.01	91.3	5.34	5.34	27.5	13	3.08	3.08	60
5	54	2.05	95.16	4.87	5.06	20	13.7	3.81	4.07	67.5
6	45	2.11	74.2	4.06	4.3	30	12	2.67	2.7	72.5
7	42	2.33	90	4.3	4.57	25	15	3.58	3.79	57.5
8	44	2.7	78	4.75	5.11	22.5	14	3.23	4.1	92.5
9	34	2.46	93	4.15	4.8	17.5	17.1	4.1	4.2	62.5
10	47	2.34	93	5.67	6.13	27.5	10.34	4.09	4.51	50
11	52	2.4	72	3.93	4.07	20	17	2.98	3.02	77.5
12	35	2.92	79.68	4.18	4.44	25	7.8	3.07	3.11	55
13	45	2.96	75	3.33	3.56	22.5	12.06	2.46	2.74	82.5
14	54	1.93	90.62	4.43	4.53	32.5	13.39	3.71	3.72	62.5
15	51	2.03	84.1	5.38	5.55	27.5	16	3.04	3.4	75

Table 4: MASTER CHART 4 GROUP IIIA (SMOKERS- SRP ALONE)

S. NO	AGE	PI	GBI1 (%)	PPD1 (mm)	CAL1 (mm)	SOD1 U/ml	GIB2 (%)	PPD2 (mm)	CAL2 (mm)	SOD2 U/ml
1	49	2.11	16	4.07	4.4	12.5	14	2.67	2.7	15
2	52	2.33	16.7	4.2	4.57	10	12	3.58	3.78	10
3	40	2.7	28.12	4.7	5.11	15	14	3.8	3.79	15
4	37	2.4	18.5	4.1	4.8	12.5	14	4	4.2	12.5
5	58	2.3	43.1	5.6	6.13	10	12	4.4	4.9	10
6	49	2.96	18.2	3.34	4	17.5	12	2.4	3	20
7	45	2.82	39.6	4.18	4.44	15	7.8	3	3.2	15
8	35	2.96	18.2	3.34	4	17.5	12	2.4	3	17.5
9	49	2.93	40	4.34	4.53	15	13.28	3.72	3.9	17.5
10	39	2.3	43	5.38	5.4	15	16	3.7	3.8	12.5
11	38	2.6	35.2	5.33	5.33	12.5	11	3.05	3.55	12.5
12	39	2.03	18.9	4.9	5.11	12.5	8.3	3.29	3.5	12.5
13	42	1.84	29	5.43	5.52	12.5	15.6	3.17	3.17	15
14	40	1.85	33	4.03	4.19	10	15	3.2	3.5	10
15	35	2.13	18	4.95	5.02	12.5	13	3.11	3.28	12.5

Table 5: MASTER CHART 5 GROUP IIIB (SMOKERS- SRP + VITAMIN C)

S. NO	AGE	PI	GBI (%)	PPD (mm)	CAL (mm)	SOD1 U/ml	GBI2 (%)	PPD2 (mm)	CAL2 (mm)	SOD2 U/ml
1	45	2.47	52	4.35	4.49	12.5	13.28	3.14	3.24	25
2	35	2.25	24	4.4	4.4	15	17.6	3.21	3.21	22.5
3	38	1.91	40	4.5	4.6	12.5	19.2	3.34	3.24	32.5
4	43	1.85	25	5.2	5.8	10	20.4	4.29	4.69	22.5
5	47	1.69	33.33	5.5	5.5	15	13.5	3.53	4.8	27.5
6	39	2.68	49.13	5.67	6.3	12.5	17.6	3.44	4.2	35
7	46	2.29	24.4	4.32	5	17.5	18	3.15	3.62	32.5
8	53	2.85	38.7	5	5.32	10	16.67	3.34	3.88	25
9	38	2.44	14.53	4.91	5.12	12.5	10.2	2.9	3.4	30
10	37	1.88	41.34	5.33	6.2	10	14.89	3.12	3.38	20
11	53	2	25	6.21	6.74	15	13.63	3.88	4.17	25
12	48	2.11	20	6.38	6.71	15	14	3.96	4.23	37.5
13	37	2	39	4.89	5.1	20	12	3.48	4	45
14	48	2	21.3	5.34	5.61	15	12.32	3.08	3.23	32.5
15	35	2.06	25.16	4.87	5.06	20	14	3.33	4.07	43

Table 6: COMPARISON OF AGE BETWEEN GROUP I,II AND III

Age (in years)	Group I		Group II		Group III		P value
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	0.447
	41.5	5.77	44.3	6.417	42.96	6.44	

Statistical Analysis: one way ANOVA

Table 7: COMPARISON OF PLAQUE INDEX BETWEEN GROUP I, II & III

Plaque Index	Group I		Group II		Group III		P value
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**0.00****
	0.246	0.229	2.204	0.335	2.291	0.376	

Statistical Analysis: one way ANOVA; **Significant at p<0.01

Table8: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP I, II AND III

	Group I		Group II		Group III		Significance
	Mean	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	
GBI (%)	15.37	7.812	86.55	6.73	29.613	10.862	0.00**
PD (mm)	1.6430	0.2941	4.87	0.805	4.825	0.736	0.00**
CAL (mm)	0.00	0.00	5.10	0.742	5.150	0.747	0.00**
SOD (U/ml)	149.2	10.277	25	3.993	13.75	2.842	0.00**

Statistical Analysis: one way ANOVA; **Significant at p<0.01

Table 9: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP II AND III

	Group II		Group III		Significance
	Mean	**S.D**	**Mean**	**S.D**	
GBI (%)	86.55	6.73	29.613	10.862	0.00**
PD (mm)	4.87	0.805	4.825	0.736	0.798
CAL (mm)	5.10	0.742	5.150	0.747	0.802
SOD (U/mI)	25	3.993	13.75	2.842	0.00**

Statistical Analysis: Independent t Test; **Significant at p<0.01

Table 10: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP II-A AND GROUP II-A_1

	Group II – A		GROUP II-A_1		Significance
	Mean	**S.D**	**Mean**	**S.D**	
GBI (%)	8.394	4.467	15.704	3.2	0.00**
PD (mm)	4.962	0.759	3.265	0.332	0.00**
CAL (mm)	5.136	0.5386	3.441	0.472	0.00**
SOD (U/mI)	25.5	4.03	25.667	3.94	0.337

Statistical Analysis: Paired t test; **Significant at p<0.01

Table 11: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP II-B AND GROUP II-B_1

	Group II - B		GROUP II-B_1		Significance
	Mean	**S.D**	**Mean**	**S.D**	
GBI (%)	84.71	8.177	13.15	2.55	0.00**
PD (mm)	4.791	0.867	3.385	0.524	0.00**
CAL (mm)	5.067	0.9177	3.616	0.591	0.00**
SOD (U/mI)	24.5	4.03	67	12.47	0.00**

Statistical Analysis: Paired t test; **Significant at p<0.01

Table12: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP III-A AND GROUP III-A_1

	Group III- A		GROUP III-A_1		Significance
	Mean	**S.D**	**Mean**	**S.D**	
GBI (%)	27.701	10.50	12.65	2.364	0.00**
PD (mm)	4.526	0.727	3.299	0.513	0.00**
CAL (mm)	4.836	0.6103	3.551	0.548	0.00**
SOD (U/mI)	13.33	2.43	13.833	2.96	0.189

Statistical Analysis: Paired t test; **Significant at p<0.01

Table13: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP III-B AND GROUP III-B_1

	Group III – B		GROUP III-B_1		Significance
	Mean	**S.D**	**Mean**	**S.D**	
GBI (%)	31.52	11.2	15.15	2.92	0.00**
PD (mm)	5.124	0.634	3.412	0.374	0.00**
CAL (mm)	5.4633	0.757	3.824	0.538	0.00**
SOD (U/mI)	14.1667	3.227	30.366	7.491	0.00**

Statistical Analysis: Paired t test; **Significant at p<0.01

Table14: COMPARISON OF SALIVARY SOD LEVEL BETWEEN GROUP II-A_1 AND GROUP II-B_1

	GROUP II-A_1		GROUP II-B_1		Significance
	Mean	**S.D**	**Mean**	**S.D**	
SOD (U/mI)	25.66	3.94	67	12.471	0.00**

Statistical Analysis: Independent t Test; **Significant at p<0.01

Table15: COMPARISON OF SALIVARY SOD LEVEL BETWEEN GROUP III-A_1 AND GROUP III-B_1

	GROUP III-A_1		GROUP III-B_1		Significance
	Mean	**S.D**	**Mean**	**S.D**	
SOD (U/mI)	13.833	2.968	30.667	7.491	0.00**

Statistical Analysis: Independent t Test; **Significant at p<0.01

Table16: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP I AND GROUP II-A_1

Values	GROUP I		GROUP II-A_1		P value
	Mean	**SD**	**Mean**	**SD**	
GBI(%)	15.3700	7.81211	15.7040	3.2960	0.018
PPD (mm)	1.6430	.29413	3.2653	.3329	.754
CAL (mm)	0.0000	0.0000	3.4413	0.4726	0.002**
SOD (U/ml)	149.25	10.277	25.666	3.949	0.000**

Statistical Analysis: Independent t Test; **Significant at p<0.01

Table17: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP I AND GROUP II-B_1

Values	GROUP I		GROUP II-B_1		P value
	Mean	**SD**	**Mean**	**SD**	
GBI (%)	15.3700	7.81211	13.1593	2.55218	0.006
PPD (mm)	1.6430	.29413	3.3853	.52403	.0130
CAL (mm)	0.0000	0.0000	3.6160	0.59185	0.000**
SOD (U/ml)	149.25	10.277	67	12.47140	0.460

Statistical Analysis: Independent t Test; **Significant at p<0.01

Table18: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP I AND GROUP III-A_1

Values	GROUP I		GROUP III-A_1		P value
	Mean	SD	Mean	SD	
GBI (%)	15.3700	7.81211	12.6653	2.36475	.004**
PPD (mm)	1.6430	.29413	3.2993	.57304	.065
CAL (mm)	0.0000	0.0000	3.5513	.54855	.001**
SOD (U/ml)	149.25	10.277	13.8333	2.96808	.000**

Statistical Analysis: Independent t Test; **Significant at p<0.01

Table19: COMPARISON OF CLINICAL PARAMETERS AND SALIVARY SOD LEVEL BETWEEN GROUP I AND GROUP III-B_1

Values	GROUP I		GROUP III-B_1		P value
	Mean	SD	Mean	SD	
GBI (%)	15.3700	7.81211	15.1527	2.92866	.014
PPD (mm)	1.6430	.29413	3.4127	0.37490	.615
CAL (mm)	0.0000	0.0000	3.8240	.53809	.000**
SOD (U/ml)	149.25	10.277	30.3667	7.49158	.137

Statistical Analysis: Independent t Test; **Significant at p<0.01

Table20: CORRELATION BETWEEN THE SALIVARY SODS LEVEL AND THE CLINICAL PARAMETERS OF GROUP I, II AND III

SOD (U/ml)		PI	GBI(%)	PPD(mm)	CAL (mm)
Group I	**Pearson correlation**	-0.248	-0.164	-0.087	0
	Sig 2 tailed	0.490	0.651	0.810	0
Group II	**Pearson correlation**	-0.140	0.024	0.072	0.069
	Sig 2 tailed	0.462	0.900	0.706	0.717
Group III	**Pearson correlation**	0.159	-0.150	-0.166	-0.184
	Sig 2 tailed	0.403	0.430	0.381	0.330

Table21: CORRELATION BETWEEN THE SALIVARY SODS LEVEL AND THE CLINICAL PARAMETERS OF GROUP I, II AND III

SOD(U/ml)		GBI(%)	PPD(mm)	CAL(mm)
Group IIA	**Pearson correlation**	-0.243	-0.061	-0.152
	Sig 2 tailed	0.382	0.828	0.589
Group II B	**Pearson correlation**	0.420	-0.448	-0.230
	Sig 2 tailed	0.119	0.094	0.409
Group IIIA	**Pearson correlation**	-0.060	-0.577	-0.541
	Sig 2 tailed	0.831	0.024	0.037
Group III B	**Pearson correlation**	-0.293	-0.013	0.148
	Sig 2 tailed	0.289	0.962	0.600

Figure 3 : Comparison of Age (in years) between Group I , Group II and Group III

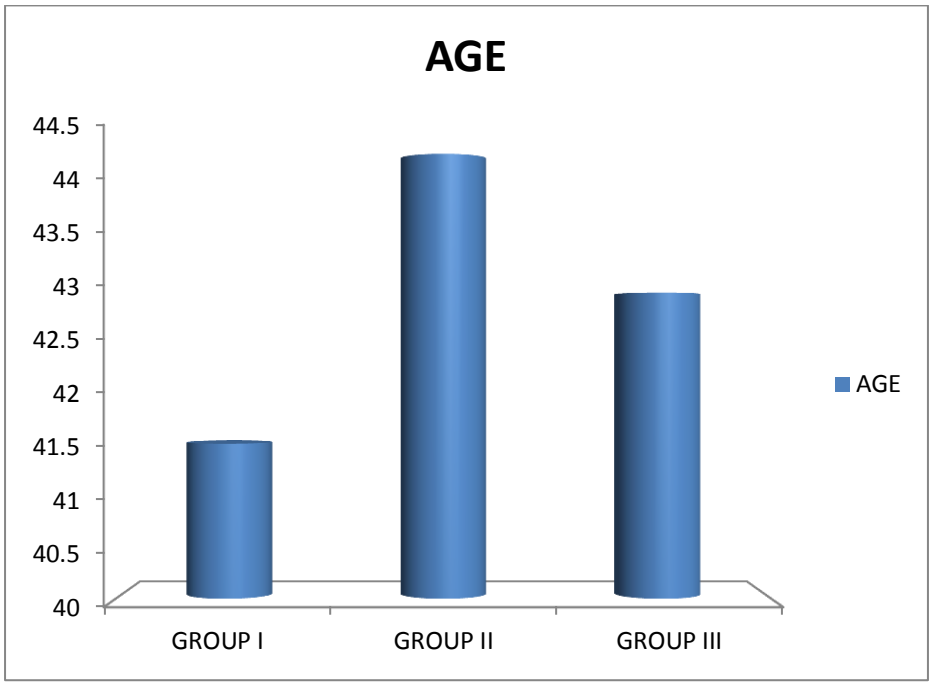


Figure 4 : Comparison of Plaque Index between Group I , Group II and Group III

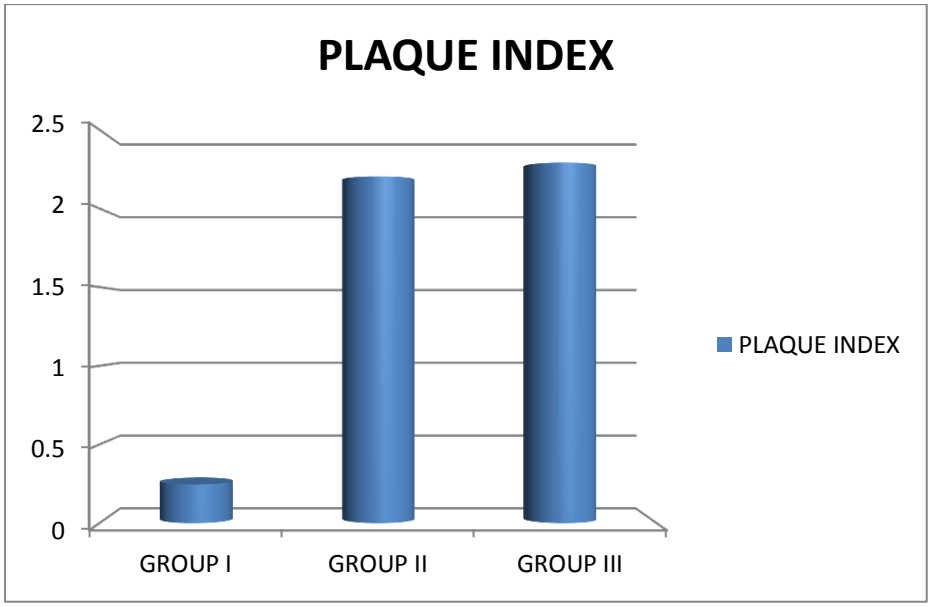


Figure 5 : Comparison of Gingival Bleeding Index between Group I , Group II A, Group II B , Group III A and Group III B

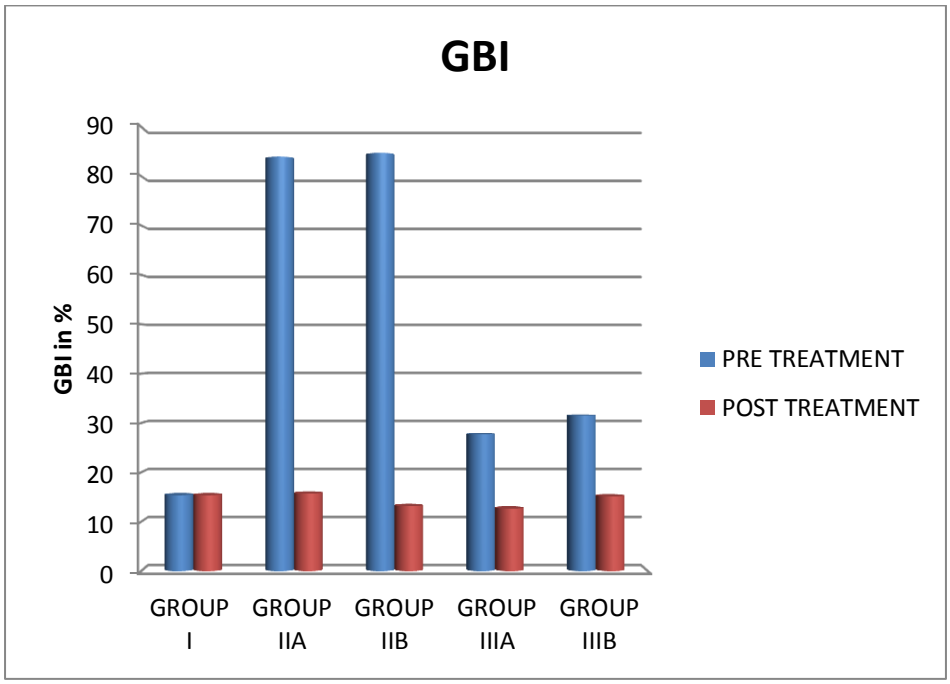


Figure 6 : Comparison of Probing Depth(in mm) between Group I , GroupIIA, Group II B, Group III A and Group III B

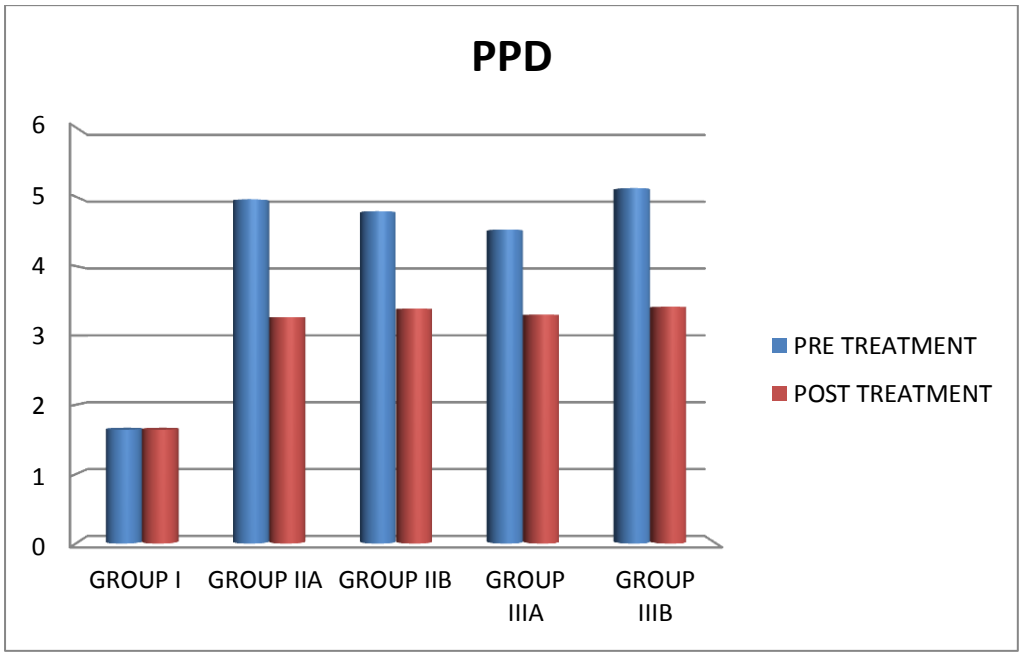


Figure 7 : Comparison of Clinical Attachment Level (in mm) between Group I , Group II A , Group II B, Group III A and Group III B

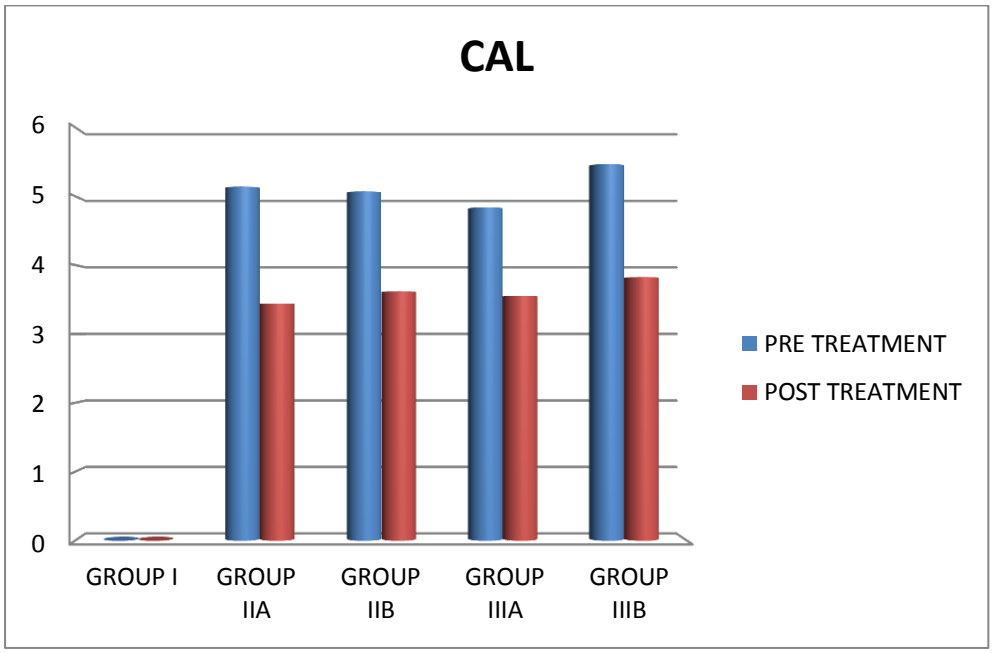


Figure 8 : Comparison of SOD Levels between GroupI , GroupII A Group II B, Group III A and Group III B

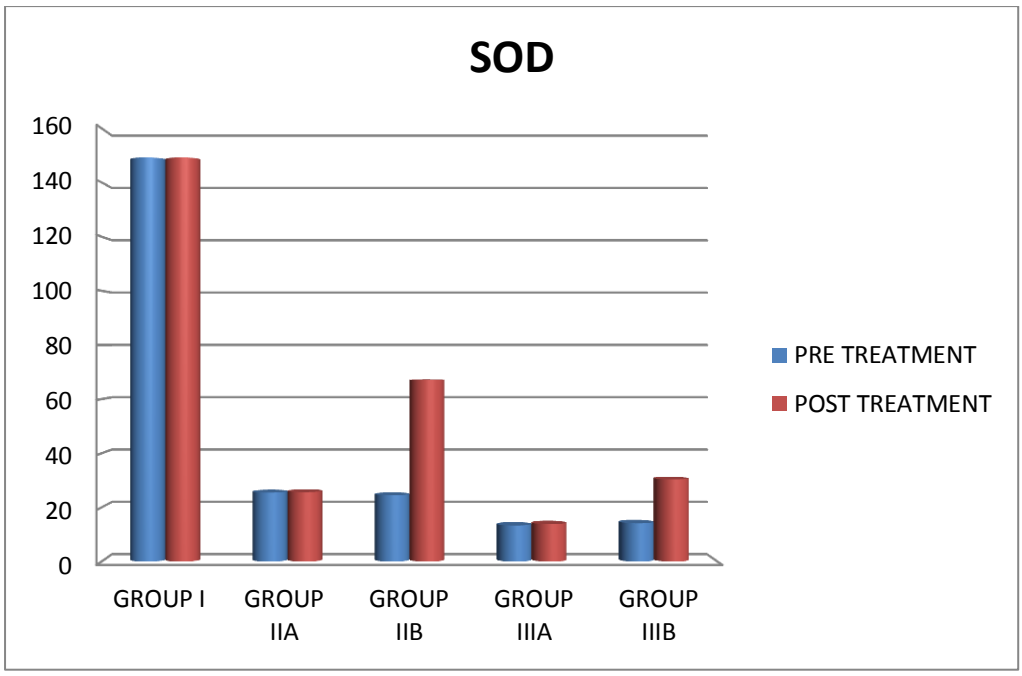


Figure 9 : Correlation Of Salivary SOD Levels And Gingival Bleeding Index In Group I

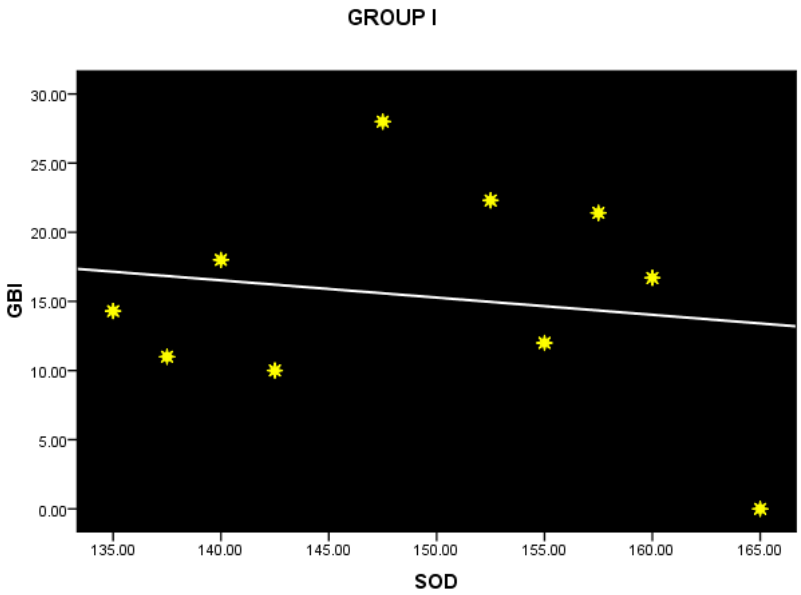


Figure 10 : Correlation Of Salivary SOD Levels And Probing Depth in Group I

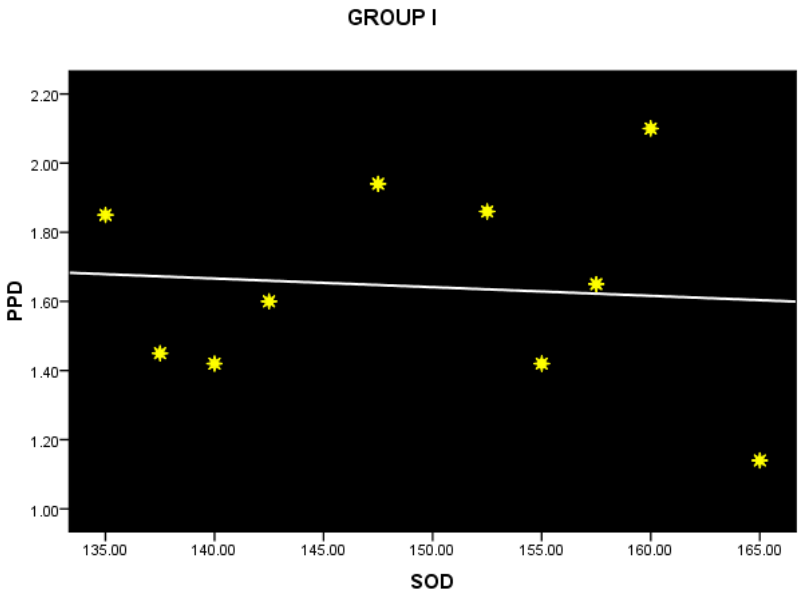


Figure 11 : Correlation Of Salivary SOD Levels And Clinical Attachment Level In Group I

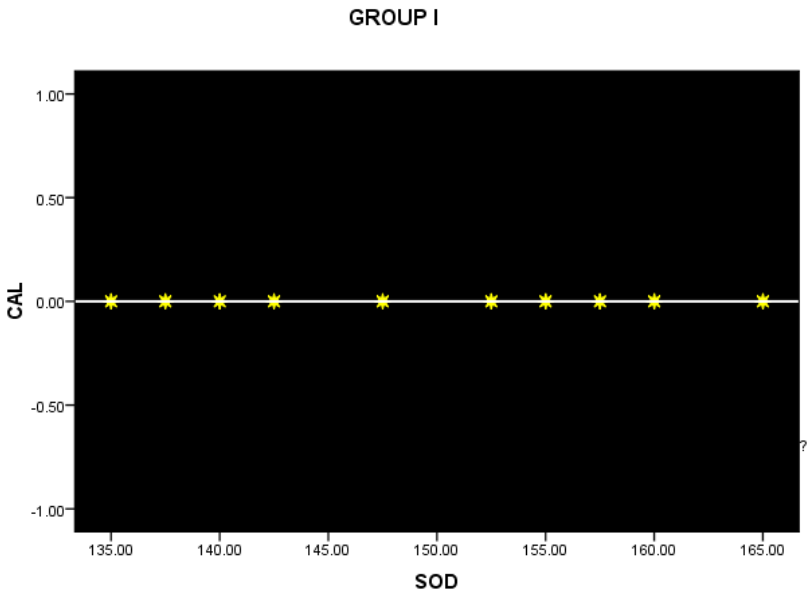


Figure 12 : Correlation Of Salivary SOD Levels And Gingival Bleeding Index In Group II A (Pre therapy)

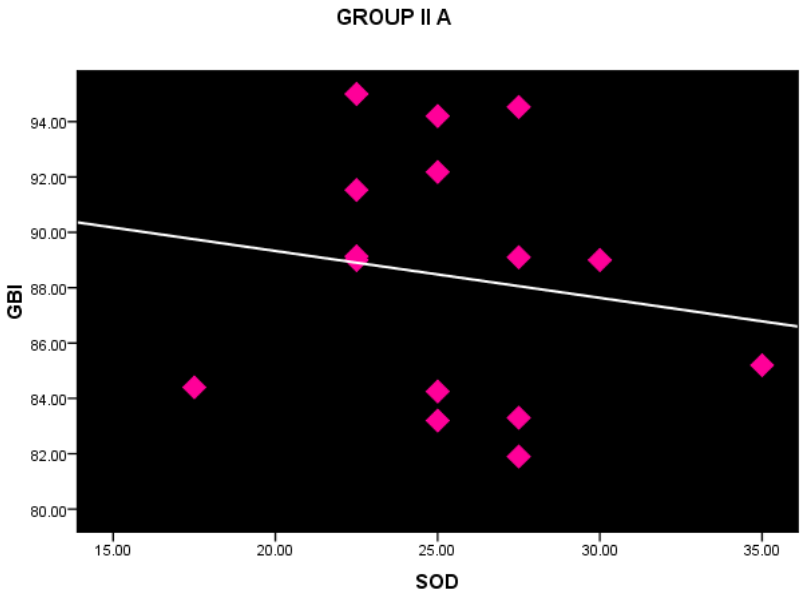


Figure 13 : Correlation Of Salivary SOD Levels And Probing Depth In Group II A (Pre therapy)

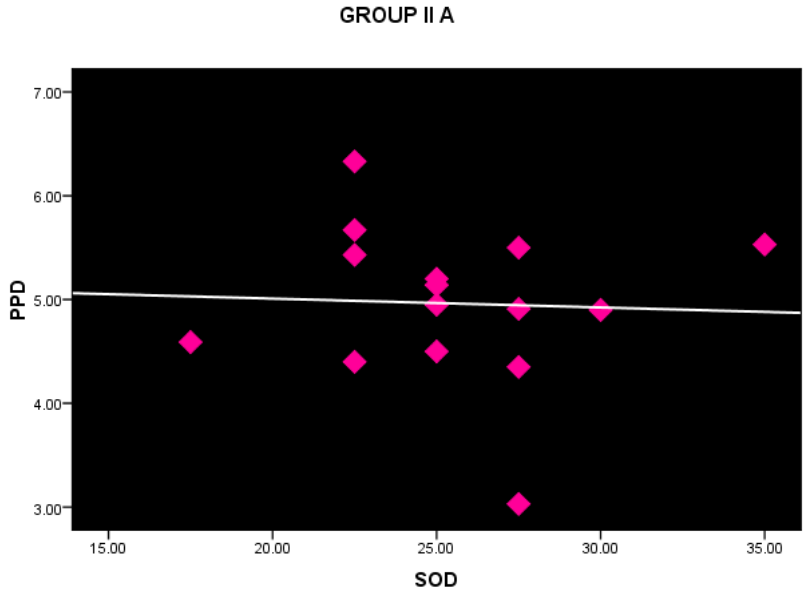


Figure 14 : Correlation Of Salivary SOD Levels And Clinical Attachment Level In Group II A (Pre therapy)

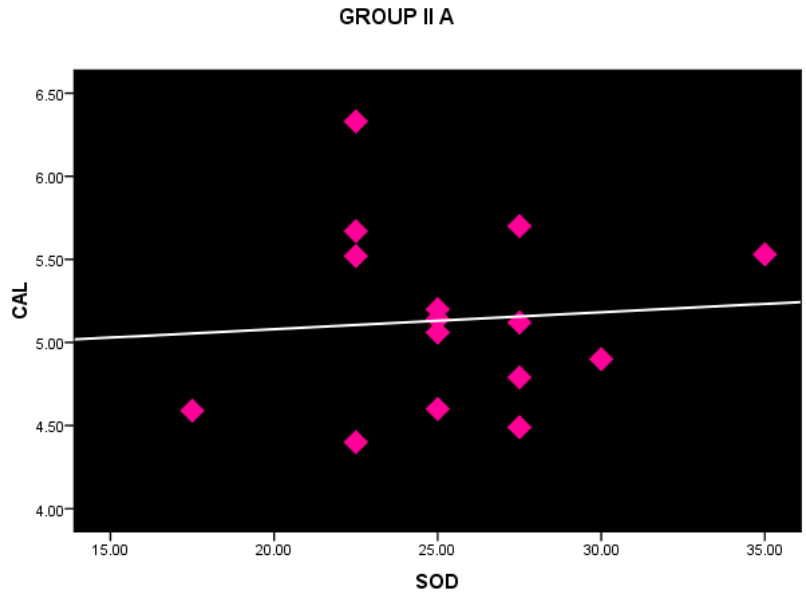


Figure 15 : Correlation Of Salivary SOD Levels And Gingival Bleeding Index In Group II B (Pre therapy)

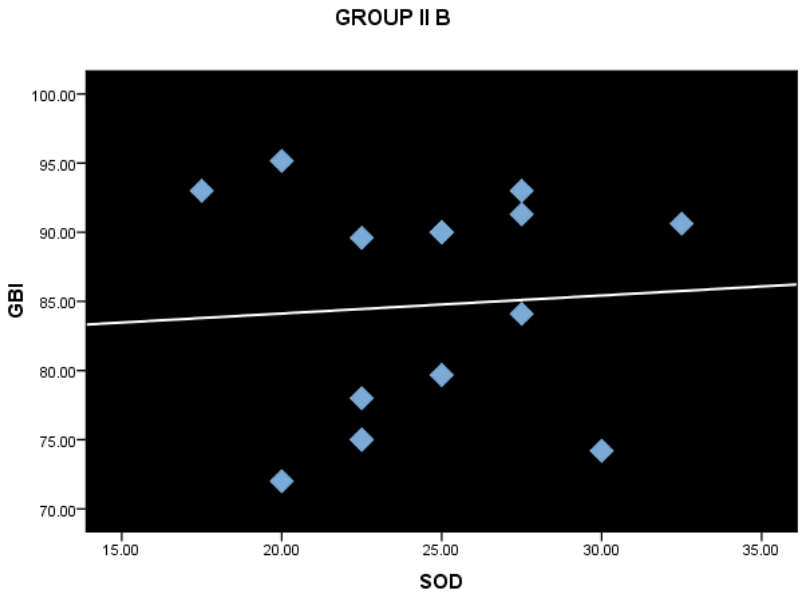


Figure 16 : Correlation Of Salivary SOD Levels And Probing Depth In Group II B (Pre therapy)

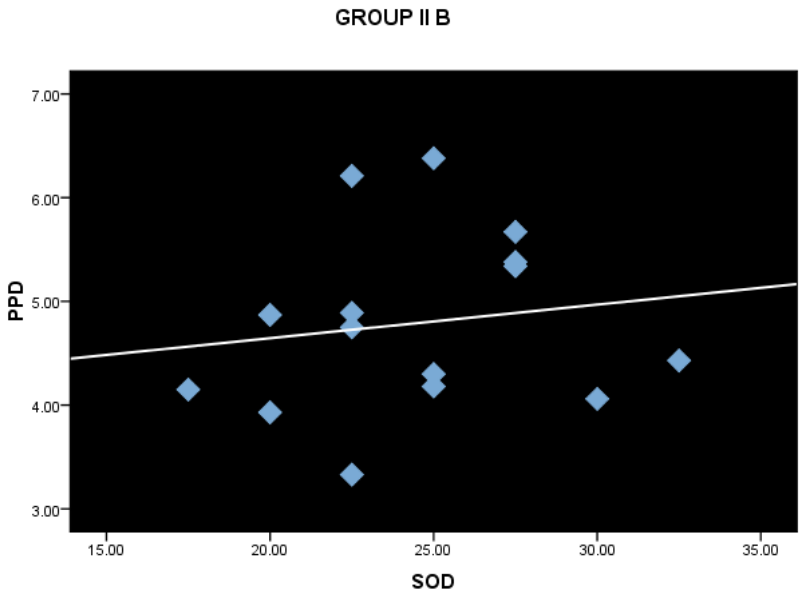


Figure 17 : Correlation Of Salivary SOD Levels And Clinical Attachment Level In Group II B (Pre therapy)

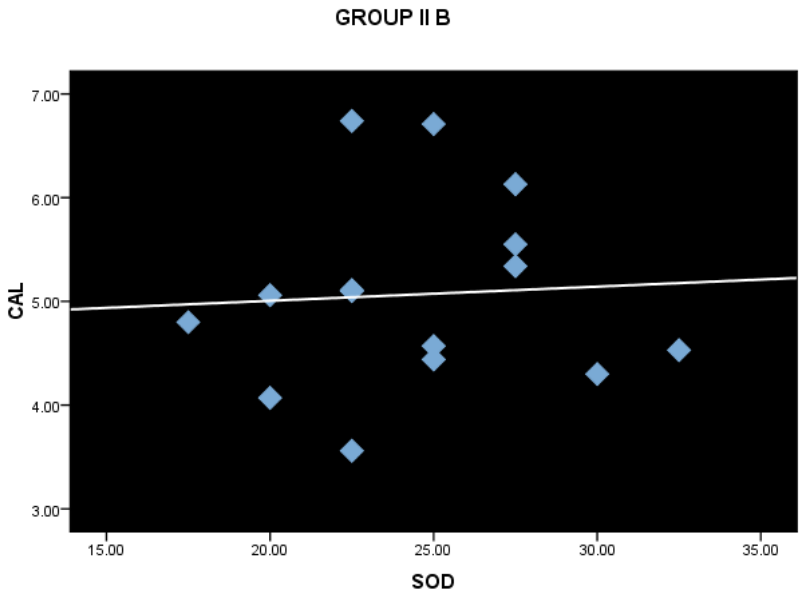


Figure 18 : Correlation Of Salivary SOD Levels And Gingival Bleeding Index In Group III A(Pre therapy)

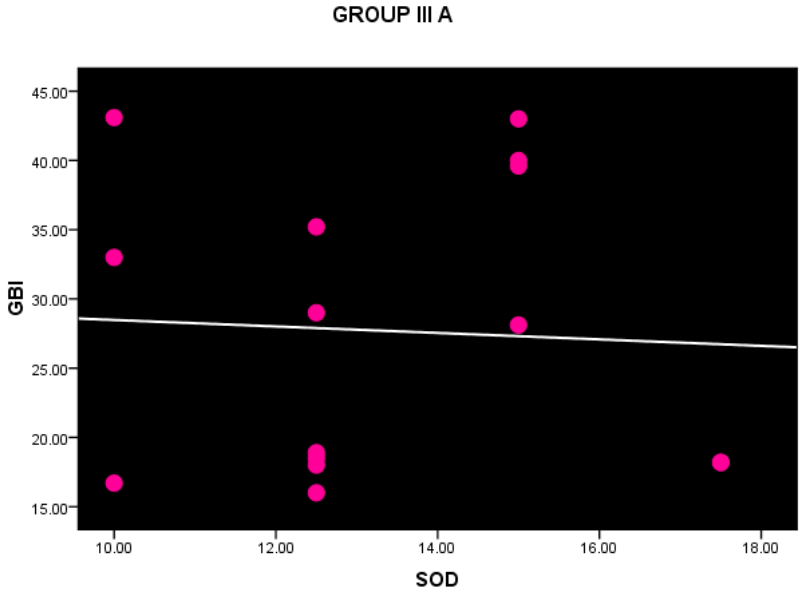


Figure 19 : Correlation Of Salivary SOD Levels And Probing Depth In Group III A (Pre therapy)

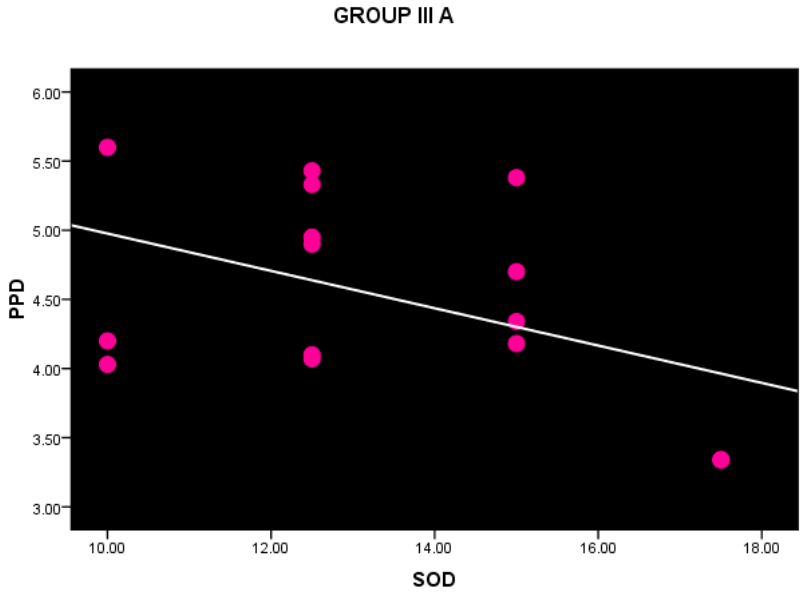


Figure 20 : Correlation Of Salivary SOD Levels And Clinical Attachment Level In Group III A (Pre therapy)

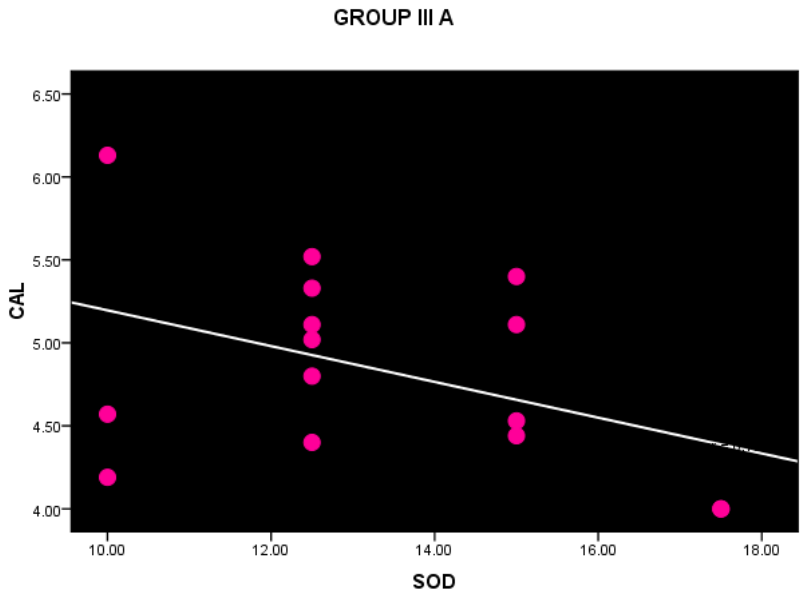


Figure 21 : Correlation Of Salivary SOD Levels And Gingival Bleeding Index In Group III B (Pre therapy)

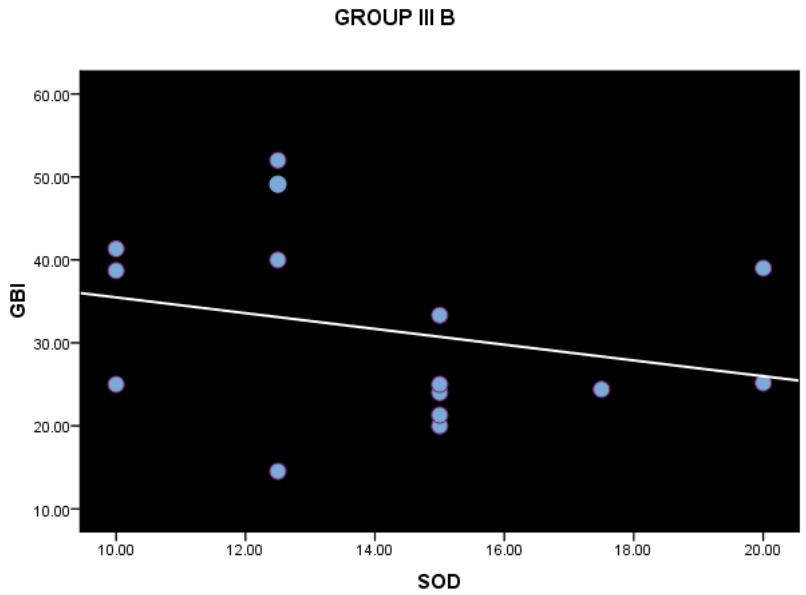


Figure 22 : Correlation Of Salivary SOD Levels And Probing Depth In Group III B (Pre therapy)

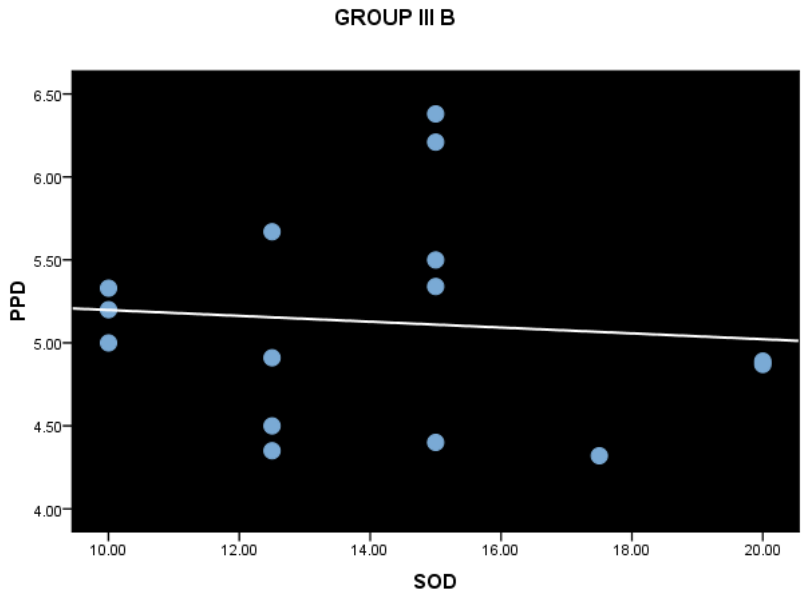


Figure 23 : Correlation Of Salivary SOD Levels And Clinical Attachment Level In Group III B (Pre therapy)

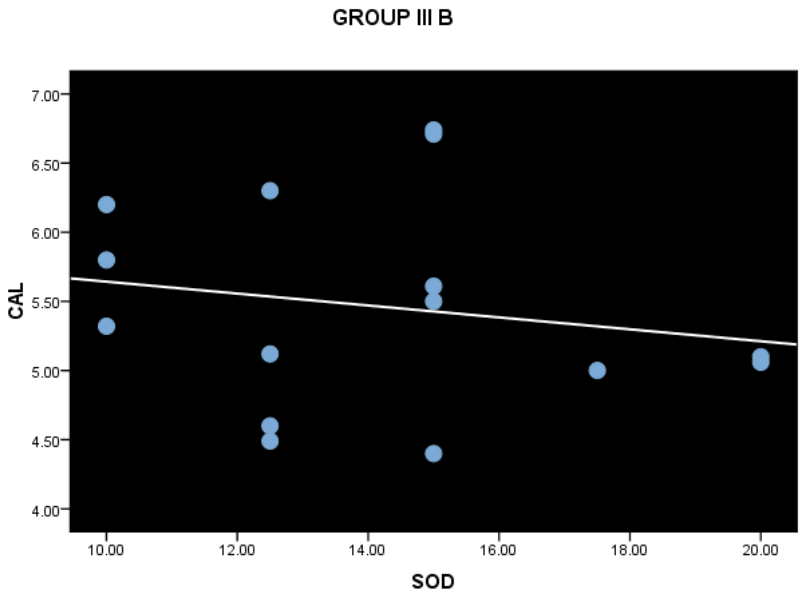


Figure 24 : Correlation Of Salivary SOD Levels And Gingival Bleeding Index In Group II A (Post therapy)

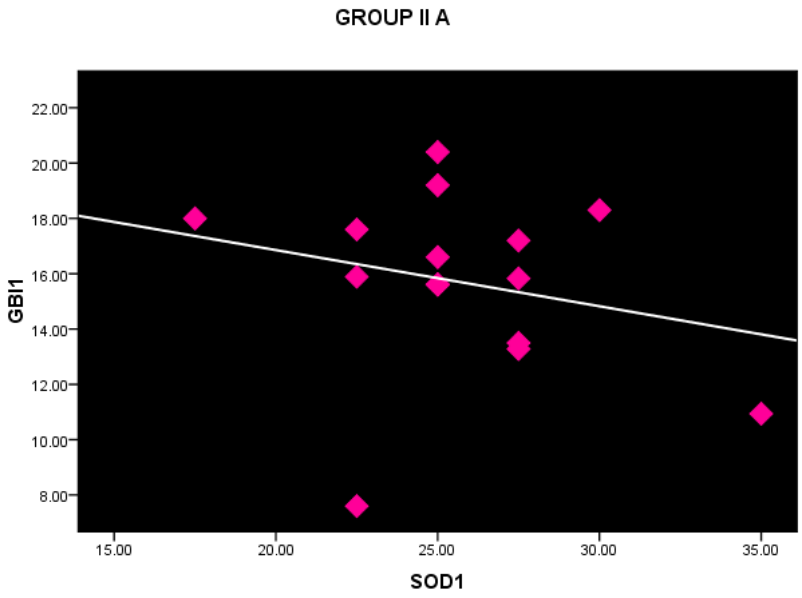


Figure 25 : Correlation Of Salivary SOD Levels And Probing Depth In Group II A (Post therapy)

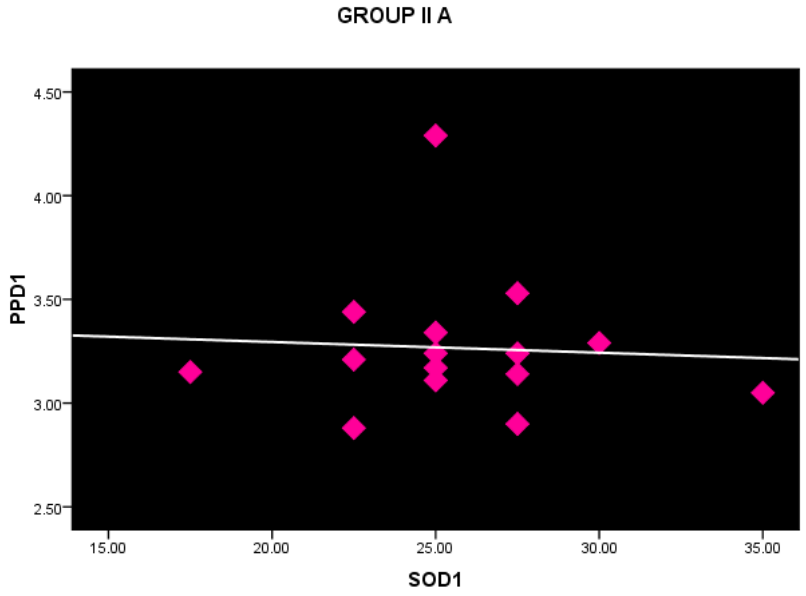


Figure 26 : Correlation Of Salivary SOD Levels And Clinical Attachment Level In Group II A (Post therapy)

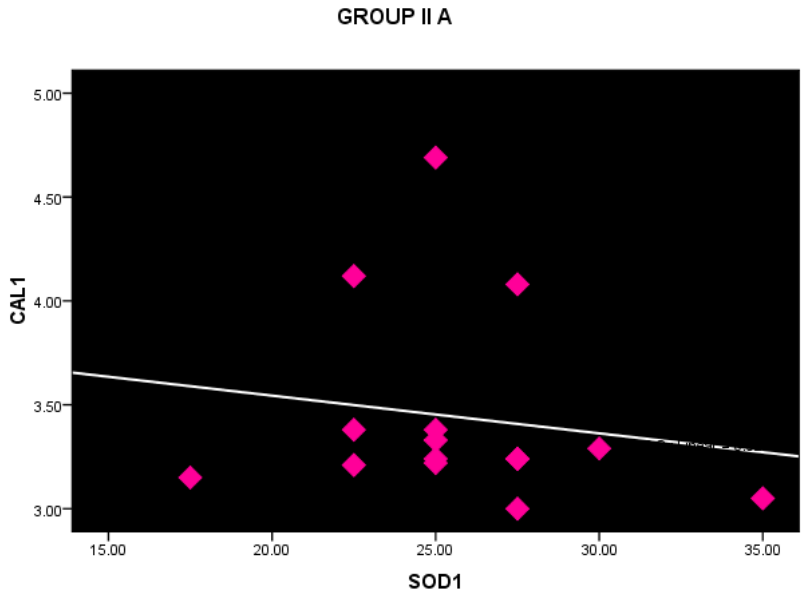


Figure 27 : Correlation Of Salivary SOD Levels And Gingival Bleeding Index In Group II B (Post therapy)

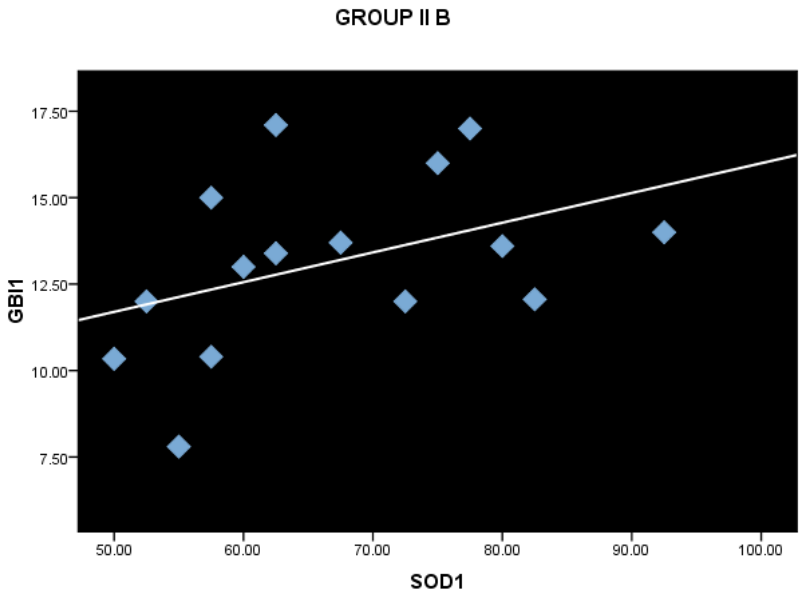


Figure 28 : Correlation Of Salivary SOD Levels And Probing Depth In Group II B (Post therapy)

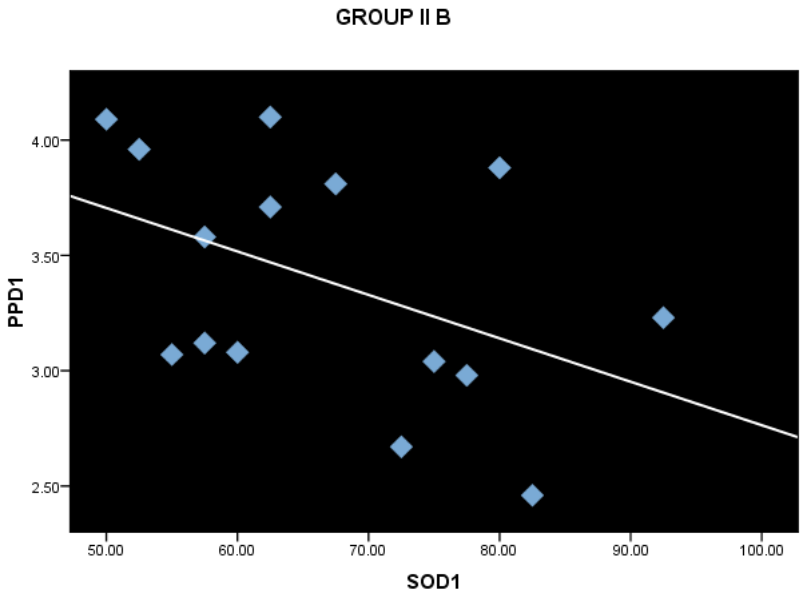


Figure 29 : Correlation Of Salivary SOD Levels And Clinical Attachment Level In Group II B (Post therapy)

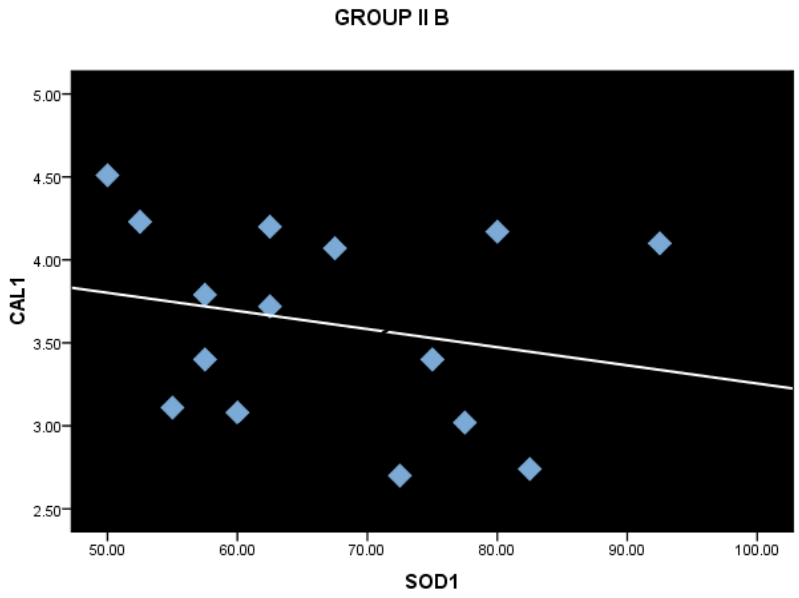


Figure 30 : Correlation Of Salivary SOD Levels And Gingival Bleeding Index In Group III A (Post therapy)

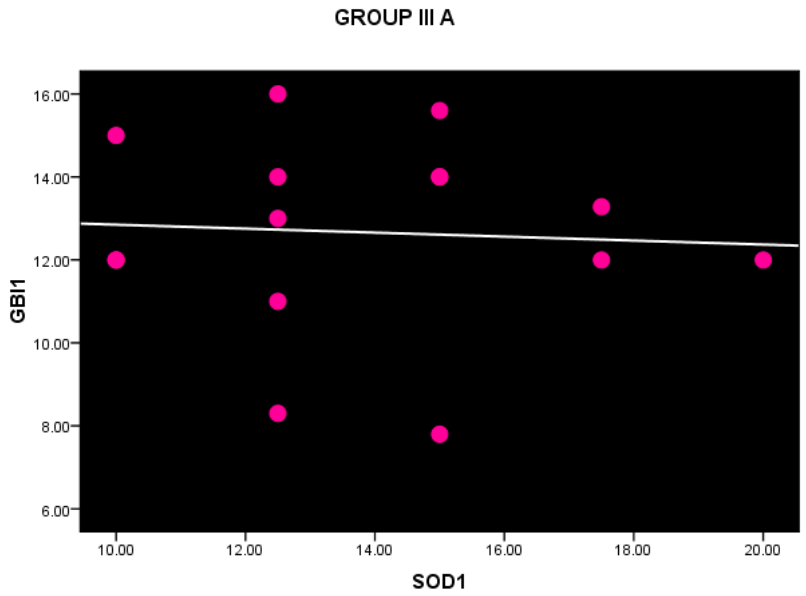


Figure 31 : Correlation Of Salivary SOD Levels And Probing Depth In Group III A (Post therapy)

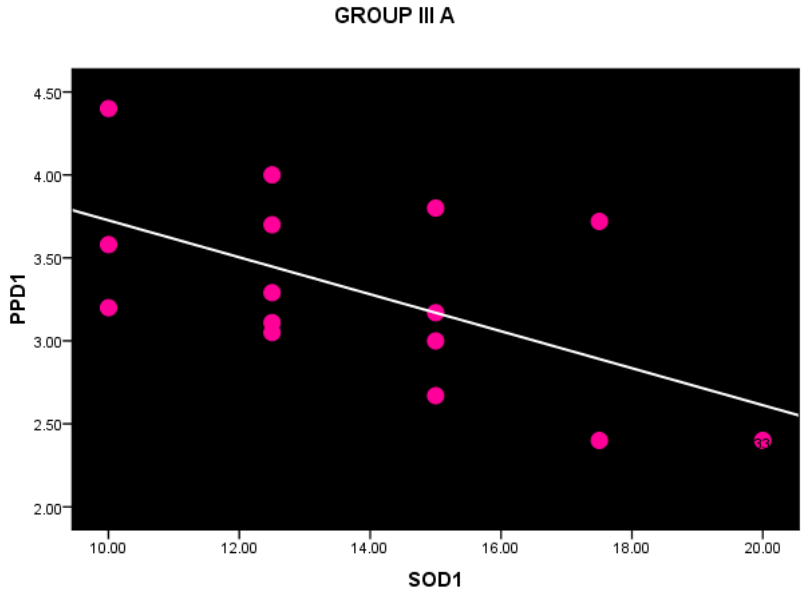


Figure 32 : Correlation Of Salivary SOD Levels And Clinical Attachment Level In Group III A (Post therapy)

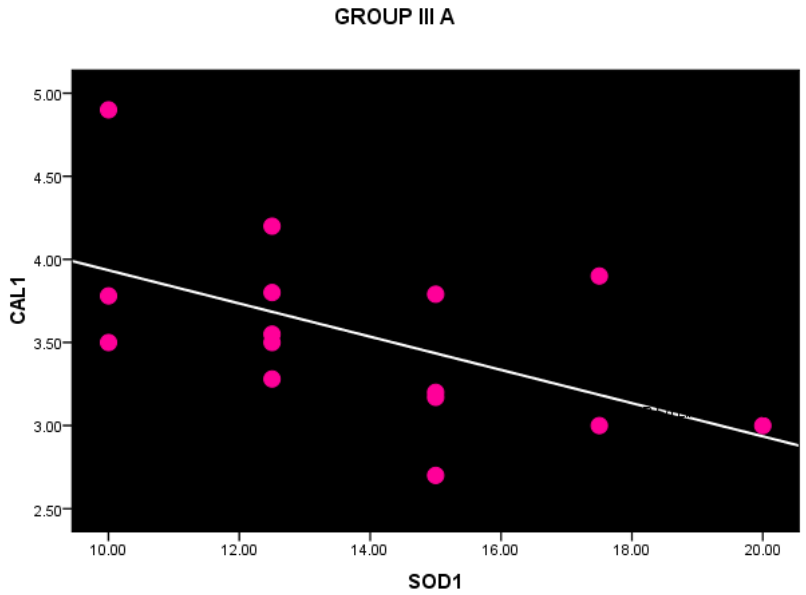


Figure 33 : Correlation Of Salivary SOD Levels And Gingival Bleeding Index In Group III B (Post therapy)

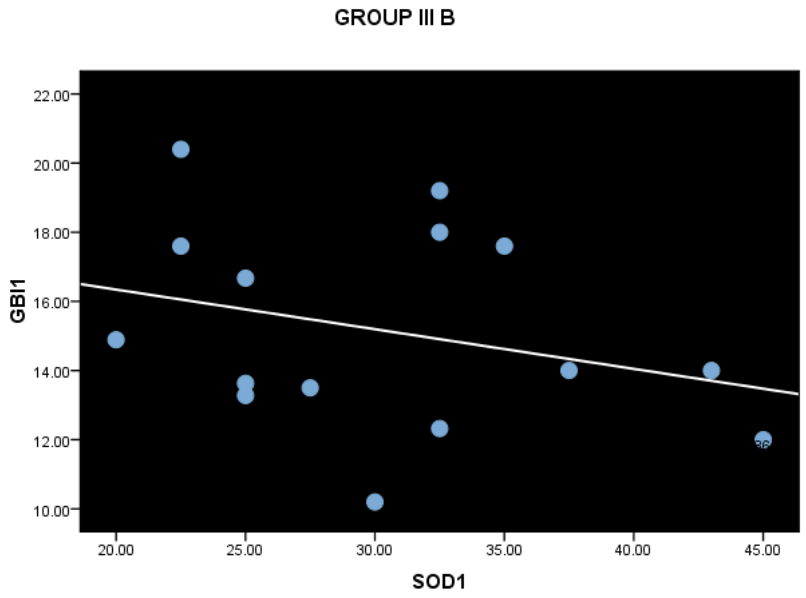


Figure 34 : Correlation Of Salivary SOD Levels And Probing Depth In Group III B (Post therapy)

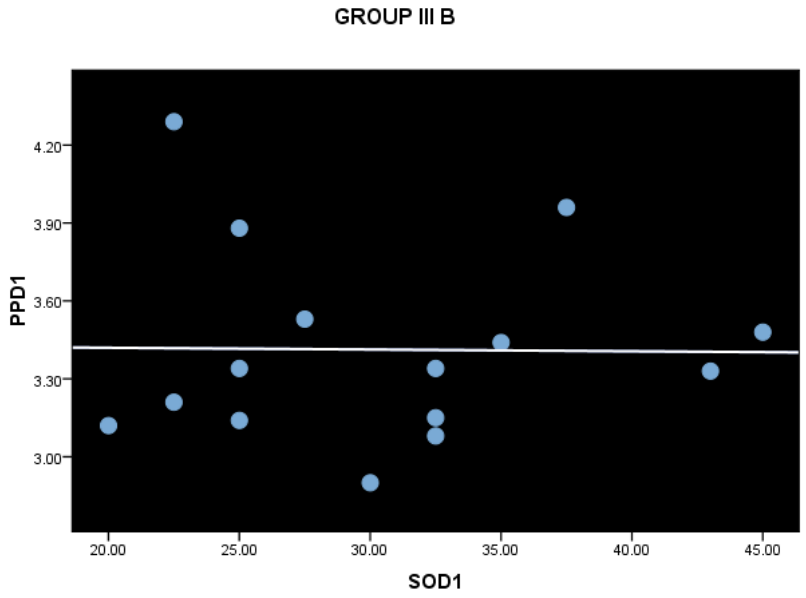
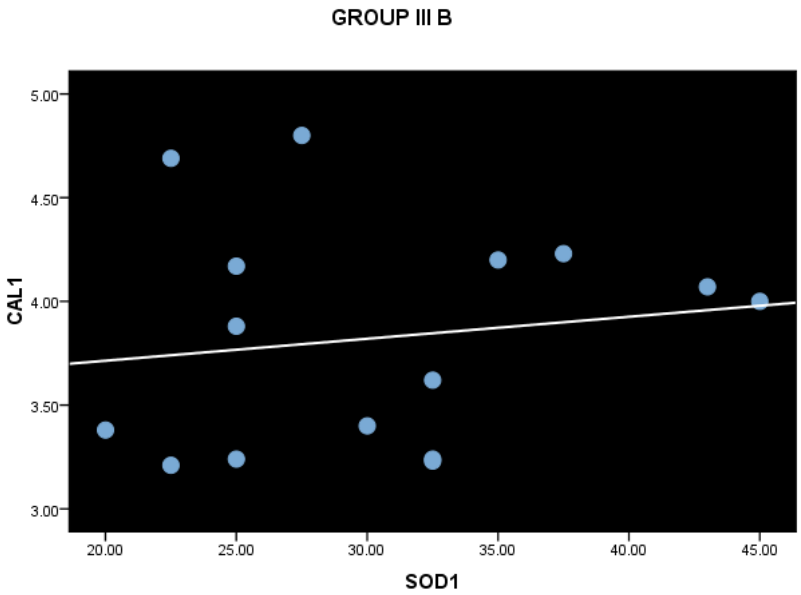


Figure 35 : Correlation Of Salivary SOD Levels And Clinical Attachment Level In Group III B (Post therapy)



DISCUSSION

Recently there is categorical evidence in the literature to identify smoking as a major risk factor for periodontitis[15,70]. Also there has been increasing interest in ROS and antioxidant system in the etiology of periodontitis.[29,33]

However, the exact mechanism how smoking exerts its destruction on periodontium is still unclear. It has been attributed that there is induction of oxidative stress by nicotine which may lead to depletion of antioxidants and this has been considered as one of the mechanisms for periodontal tissue damage.

Many studies in the periodontal field have been carried out in GCF, serum and very recently in saliva to find out antioxidant conditions of periodontitis patients. In this study whole saliva was used as sampling medium. In contrast to GCF whole saliva is a pool of all diseased sites of oral cavity and method of collection is non-invasive as well as easy to perform.[64]

The main finding in the present study was, baseline salivary SOD level significantly reduced in smokers than non-smokers with chronic periodontitis (ChP) as compared to control group. There was a significant reduction in all clinical parameters in smokers and non-smokers with ChP one month following SRP. Salivary SOD level significantly increased ($p<0.01$)in smokers and non-smokers with ChP one month following SRP supplemented with vitamin C.

In the present study at baseline, all the clinical parameters namely PI, GBI, PD and CAL were significantly higher in non-smokers and smokers with ChP as compared to the periodontally healthy controls (p value <0.01) *(table 8)*. There was

negative correlation between salivary SOD and all clinical parameters in group I *(table 20)*.

The baseline gingival bleeding index was significantly reduced ($p < 0.01$) in smokers with ChP group as compared to non-smokers with ChP group but GBI score was significantly higher in both the groups than control group with a p value <0.01 *(table 8 and 9)*. This finding is in accordance with the concept that, smokers generally present with reduced gingival inflammation and bleeding on probing as compared to non-smokers, because of strong, chronic, dose-dependent suppressive effect of smoking on gingival inflammation and bleeding as documented in the Third National Health and Nutritional Examination Survey (NHANES III). This is also in agreement with findings of *Nair P et al 2003* who emphasised the effect of smoking in masking the signs and symptoms of inflammation in periodontal disease.[53]

SOD is an intrinsic AO as well as a primary defending substance that protects cells from ROS by converting superoxide anion into H_2O_2. Several studies have examined the relationship between SOD and periodontitis.[4,13,64]

The present study shows statistically significant difference (p value <0.01) in baseline salivary SOD level in control, non-smokers and smokers with ChP. Salivary SOD level was observed to be least in smokers with ChP while it is maximum in healthy controls *(table 8)*. This finding is concurrent with *Agnihotri R 2009* who concluded that a progressive reduction in SOD levels in saliva and GCF in healthy non smokers to light smokers to heavy smokers with ChP.[2] *Garg et al 2006* concluded that SOD levels were higher in non-smokers than smokers both in tissue and blood and his finding is similar to the findings of the present study.[30]

The reason for the decreased salivary SOD level may be due to increased oxidative stress and nicotine in smokers that had caused the depletion of antioxidant enzymes and also by negative feedback inactivation of SOD due to over production of hydrogen peroxide.[2]

Chronic periodontitis is primarily a gram negative infection caused by putative periodontal pathogens resulting in immune-inflammatory response. Since SRP is the corner stone of periodontal therapy to reduce the bacterial burden, in the present study SRP is being opted as treatment modality.

Vitamin C is an essential dietary vitamin which cannot be synthesised by human cells. It is an electron donor and this property accounts for its antioxidant function. It is a co-factor for many enzymes and has the ability to regenerate other antioxidants.[8] Since ROS plays an important role in the pathogenesis of periodontitis, many antioxidants have been supplemented in recent researches to evaluate its effectiveness in periodontal treatment.[6,8]

In the present study we have supplemented 500mg vitamin C in tablet form once daily from the day of SRP for one month to evaluate whether it has any additional effect in improving the antioxidant level by measuring salivary SOD.

In the present study, after one month of treatment the gingival bleeding index, probing depth and clinical attachment level were significantly reduced in non-smokers (group II) and smokers (group III) with ChP *(tables 10, 11, 12 and 13)*. There was no significant difference in the clinical parameters after one month of treatment between non-smokers group IIA and smokers group IIIA (with SRP alone) as compared to non-smokers group IIB and smokers group IIIB (with SRP and vitamin C) *(tables 14 and 15)*

This implies that the adjunctive dose of vitamin C in group IIB and group IIIB did not offer any additional effect in regard to clinical parameters. This is in accordance with *Ali E et al 2010* who evaluated the effect of vitamin C as adjunct to SRP in chronic periodontitis subjects and concluded that there was no significant difference in post treatment clinical parameter between SRP group as compared to group with SRP and vitamin C.[8.] This is in concurrence to the findings of *Leggott et al* who did not find a relationship between PD and BOP measures and the concentration of ascorbic acid in periodontitis subjects after using a dose of vitamin C as an adjunct to non-surgical periodontal treatment.[44]

In the present study, salivary SOD level shows no significant difference between the baseline and post-treatment value in non-smokers (group IIA) and smokers (group III A) with SRP alone *(tables 10 and 12)*. This finding of the present study is in contrast to *Kim et al 2010* who compared the total antioxidant status (TAS) and superoxide dismutase (SOD) activity in the saliva of periodontally compromised patients before and after scaling and root planing (SRP) therapy and concluded that the SOD activity had increased at 3 months after SRP[42]. This variation in findings may be due to the difference in the time of post-treatment sampling in our study as compared to the above mentioned study.

The salivary SOD levels significantly increased ($P<0.01$) after one month of vitamin C supplementation alongwith SRP in Group II B and Group III B *(tables 11 and 13)*. However, this increase in SOD levels after treatment were still less compared to periodontally healthy controls *(tables 17 and 19)*.

SUMMARY AND CONCLUSION

In the present study 70 male subjects were recruited to evaluate and compare the salivary SOD level in smokers and non-smokers with chronic periodontitis before and after SRP with vitamin C as an adjunct.

From the results of this study the following conclusion can be drawn:

1. Baseline salivary SOD level in smokers with chronic periodontitis is less compared to periodontally healthy controls.
2. Baseline salivary SOD level in smokers with chronic periodontitis is less compared to non-smokers with chronic periodontitis.
3. SRP in smokers and non-smokers with chronic periodontitis does not affect the salivary SOD level after one month.
4. Adjunctive dose of vitamin C has improved salivary SOD level in smokers and non-smokers with chronic periodontitis than SRP alone.

In the periodontal literature it has been hypothesized that deficiency of vitamin C has been considered as a risk factor in the pathogenesis of periodontitis. Here in the present study vitamin C supplementation had resulted in a significant improvement in salivary antioxidant status in subjects with chronic periodontitis. Further longitudinal studies may be needed for the administration of vitamin C as an adjunct to periodontal therapy in order to maintain a stable periodontium.

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ANNEXURE : 1

INFORMATION SHEET

We are conducting a study on **evaluation of effectiveness of vitamin C following phase I therapy by estimating salivary superoxide dismutase level in smokers with chronic periodontitis**

The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

- Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Name of the patient Signature / Thumb impression

Name of the investigator Signature
Date

ANNEXURE : 2

INFORMED CONSENT FORM

EVALUATION OF EFFECTIVENESS OF VITAMIN C FOLLOWING PHASE I THERAPY BY ESTIMATING SALIVARY SUPEROXIDE DISMUTASE LEVEL IN SMOKERS WITH CHRONIC PERIODONTITIS

Name: Age / Sex: O.P.No :

Address:

I, _____age____years

exercising my free power of choice, hereby give my consent to be included as a participant in the study **evaluation of effectiveness of vitamin C following phase I therapy by estimating salivary superoxide dismutase level in smokers with chronic periodontitis** I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I agree to co-operate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential

Name of the patient Signature **/** Thumb impression

Name of the investigator Signature
Date

ANNEXURE : 3

ஆராய்ச்சி ஒப்புதல் கடிதம்

நாட்பட்ட ஈறு அழற்சி நோயினால் பாதிக்கப்பட்ட புகைப்பிடிக்கும் நோயாளிகளின் உமிழ்நீரில் சூப்பர் ஆக்ஸைடு டிஸ்முடேஸ் அளவினை சிகிச்சைக்கு முன்பும் பின்பும் ஆராய்ந்தறிதல்

பெயர்	:	தேதி	:
வயது	:	புற நோயாளி எண்	:
பாலினம்	:	ஆராய்ச்சி சேர்க்கை எண்	:

என்னுடைய சுய நினைவுடனும் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் சேர்த்துக்கொள்ள ஒப்புதல் அளிக்கிறேன்.

கீழ்காணப்படும் நிபந்தனைகளுக்கு நான் ஒப்புதல் அளிக்கிறேன்.

- இந்த ஆராய்ச்சியின் நோக்கமும், செயல் முறைகளும் எனக்கு திருப்தியளிக்கும் வகையில் அறிவுறுத்தப்பட்டது.
- நான் ஏற்கனவே உட்கொண்ட மற்றும் உட்கொள்கிற மருந்துகளைப் பற்றிய விபரங்கள் ஆராய்ச்சியாளரிடம் தெரிவித்துள்ளேன்.
- என் உடல்நலம் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத வழக்கத்திற்கு மாறான நோய்க்குறி தென்பட்டாலோ அதனை உடனடியாக மருத்துவரிடம் தெரிவிக்க சம்மதிக்கிறேன்.
- என் மருத்துவ குறிப்பேடுகளை இந்த ஆராய்ச்சியில் பயன்படுத்திக்கொள்ள சம்மதிக்கிறேன். இந்த ஆராய்ச்சி மையமும், ஆராய்ச்சியாளரும் என்னுடைய விவரங்கள் அனைத்தையும் இரகசியமாக வைப்பதாக அறிகிறேன்.

.....	.....	.....
நோயாளியின் பெயர்	கையொப்பம்	தேதி
.....	.....	.....
ஆராய்ச்சியாளரின் பெயர்	கையொப்பம்	தேதி

ANNEXURE : 4

EVALUATION OF EFFECTIVENESS OF VITAMIN C FOLLOWING PHASE I THERAPY BY ESTIMATING SALIVARY SUPEROXIDE DISMUTASE LEVEL IN SMOKERS WITH CHRONIC PERIODONTITIS

PROFORMA

Name : Age / Gender:

O.P. No : Code No:

Occupation : Income :

Address and Contact No.:

Chief Complaints

Duration:

Medical history

Dental history

Habits:

Periodontal Examination:

PLAQUE INDEX – SILNESS & LOE (1964)

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Score:

GINGIVAL BLEEDING INDEX – AINAMO & BAY (1975)

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Score:

PROBING DEPTH & CLINICAL ATTACHMENT LEVEL (in mm)

MAXILLARY:

Palatal

CAL																
PPD																
	18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
PPD																
CAL																

Buccal

MANDIBULAR:

Lingual

CAL																
PPD																
	48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
PPD																
CAL																

Buccal

DIAGNOSIS:

INVESTIGATIONS:

OPG

SALIVARY SOD LEVEL

<u>TREATMENT</u>

<u>AFTER THERAPY (after 1 month)</u>

GINGIVAL BLEEDING INDEX – AINAMO & BAY (1975)

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Score:

PROBING DEPTH & CLINICAL ATTACHMENT LEVEL (in mm)

MAXILLARY:

Palatal

CAL															
PPD															
	18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
PPD															
CAL															

Buccal

MANDIBULAR:

Lingual

CAL																
PPD																
	48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
PPD																
CAL																

Buccal

INVESTIGATION:

SALIVARY SOD LEVEL:

INFERENCE:

Signature of the P.G student **Signature of Guide**

Date